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Early Kidney Dysfunction in Metabolic-Associated Fatty Liver Disease: Is Transient Elastography Useful as a Screening Method?

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Keywords
Metabolic-associated fatty liver disease · Transient elastography · Fibroscan · Liver fibrosis · Early kidney dysfunction · Microalbuminuria

Abstract
Introduction: Increasing evidence suggests an association between metabolic-associated fatty liver disease (MAFLD) and CKD. Timely prediction of early kidney dysfunction (EKD) is thus essential in this population although a screening method is not stablished. We aimed to evaluate the role of transient elastography (TE) in predicting EKD in patients with MAFLD. ***Materials and Methods:*** A prospective cohort study that included patients with MAFLD scheduled for evaluation was performed between May 2019 and January 2020. Demographic, clinical, and laboratory data and TE parameters were prospectively obtained. EKD was defined as microalbuminuria (urinary albumin-to-Cr ratio 30–300 mg/g) and estimated glomerular filtration rate ≥60 mL/min/1.73 m^2. Significant liver fibrosis was defined as liver stiffness measurement (LSM) ≥8.2 kPa. ***Results:*** Of the included 45 patients with MALFD, 53.3% were of female gender with mean age of 53.5 ± 10.9 years. EKD was found in 17.8% of patients. MAFLD patients with EKD were significantly more obese (BMI ≥30) (75.0 vs. 32.4%, $p = 0.045$) and had significantly higher LSM (8.5 ± 4.1 vs. 5.8 ± 2.2 kPa, $p = 0.01$). After adjustment of potential confounders for EKD, the presence of liver fibrosis remained a significant predictor of EKD, being associated with a 14.3-fold increased risk of EKD ($p = 0.04$). The optimal cutoff value of LSM to predict EKD was 6.1 kPa (sensitivity: 85.7%; specificity: 67.6%). ***Conclusion:*** Significant liver fibrosis is associated with a significant increased risk of EKD in patients with MAFLD, regardless of other comorbidities. Higher levels of LSM, particularly >6.1 kPa, alert for timely identification of EKD and associated comorbidities, as well as their control, in order to prevent the development of CKD in the long term.

Introduction

The metabolic-associated fatty liver disease (MAFLD) is characterized by an excessive liver fat accumulation and constitutes a spectrum of clinical conditions, ranging from liver steatosis to more severe conditions such as steatohepatitis (NASH), cirrhosis, and hepatocellular carcinoma [1, 2]. It is the most common liver disorder in

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Western countries [3] and has been identified in a progressively larger percentage of the population (6–35% of adults) [3, 4], being tightly associated with insulin resistance and multiple metabolic risk factors, namely, type 2 diabetes mellitus (DM), obesity, and metabolic syndrome, which also increase the risk of more advanced disease [1].

It is now increasingly recognized that MAFLD not only damages the liver but can also increase the risk of developing extrahepatic diseases, including type 2 DM, cardiovascular disease, and CKD, which have a substantial impact on health-care resources [1, 5]. Accumulating evidence indicates that MAFLD predisposes to atherogenic dyslipidemia, exacerbates insulin resistance, and releases a variety of proinflammatory and prothrombotic factors, that can promote vascular and renal damage. Moreover, interactions between affected organs or tissues may further impair function and worsen patient outcomes [6]. CKD was described in 20–50% of MAFLD patients, particularly in biopsy-proven NASH [7], and is independently associated with increased mortality in these patients [8]. Moreover, previous studies verified that the presence of fatty liver is associated with the future decline of kidney function [9] and accelerates kidney function decline in CKD [10]. Although increasing evidence suggests an association between MAFLD and CKD [5, 11–16], whether a causal relationship exists remains to be definitively established since data are conflicting [8, 17, 18].

Timely prediction of early kidney dysfunction (EKD) is thus essential in patients with MAFLD although the screening method is not stablished.

Fibrosis is the most important prognostic factor in MAFLD and is correlated with liver-related outcomes and mortality [19]. Transient elastography (TE) is a noninvasive and reliable tool widely used for the assessment of liver stiffness measurement (LSM), giving reproducible results with the ability to accurately detect advanced liver fibrosis and cirrhosis [20–23]. Studies revealed that LSM assessed by TE was found to be a potential indicator of CKD in patients with MAFLD [24], having MAFLD patients with advanced fibrosis a higher risk of CKD [17]. Recently, Sun et al. [25], also revealed that liver fibrosis assessed by TE was independently associated with EKD in patients with biopsy-proven MAFLD.

Despite several studies regarding the association between MAFLD and CKD, there is paucity in the literature related to early detection of kidney disease and how to best manage these patients. It seems extremely important to identify patients with MAFLD at higher risk of EKD to define a risk population for surveillance and therapy intensification measures, in order to prevent long-term progression to CKD and adverse outcomes.

The aim of this study was to explore the potential value of TE in predicting EKD in patients with MAFLD.

Methods

Study Design and Patients' Selection

We performed a prospective cohort study including consecutive patients with MAFLD followed in a gastroenterology and hepatology consultation of a university-affiliated hospital and scheduled for evaluation, between May 2019 and January 2020.

MAFLD was diagnosed based on abdominal ultrasonography (hepatorenal echo contrast and liver brightness) and/or liver biopsy after exclusion of a daily alcohol consumption ≥20 g/day for women and ≥30 g/day for men [26], other potential concurrent liver disease, and medications that may cause steatosis (amiodarone, methotrexate, tamoxifen, valproic acid, antimycotic medications, or oral corticosteroids). Those also excluded were patients aged under 18 years, with CKD, patients with liver transplantation, hepatic surgery, ascites or pregnancy, patients with conditions that can interfere with LSM such as extrahepatic cholestasis, aminotransferases ≥5× upper limit of normal and right heart failure or other causes of liver congestion, patients with unreliable TE measures, and patients that refused to participate in the study.

Analyzed Variables and Definitions

Data were collected prospectively and registered anonymously in a database. Demographic, anthropometric, clinical, laboratory, and TE data were obtained. Demographic and anthropometric data included age, gender, race, weight, height, and waist circumference. The body weight (kg) and height (m) were determined with a weighing scale and measuring tape, respectively, both committed for this purpose. The BMI (kg/m^2) was calculated using body weight and height. Obesity was defined as BMI ≥30 kg/m^2. Waist circumference (cm) was determined for the definition of metabolic syndrome and was measured at a level midway between the lowest rib and the iliac crest. Clinical data included systolic and diastolic blood pressure, comorbidities, namely, arterial hypertension, dyslipidemia, type 2 DM, metabolic syndrome, cardiovascular disease (e.g., heart failure and ischemic heart disease), cerebrovascular disease, smoking status, alcohol consumption (exclusion criteria), and medication use (insulin and oral antidiabetics). The blood pressure value was determined by the average of 3 measurements. Metabolic syndrome was defined by the International Diabetes Federation criteria [27] as central obesity that corresponds to abdominal circumference ≥94 cm in men; ≥80 cm in women and at least 2 parameters of the following: triglycerides ≥150 mg/dL or specific treatment for hypertriglyceridemia; high-density lipoprotein cholesterol <40 mg/dL in men and <50 mg/dL in women or specific treatment for dyslipidemia; systolic blood pressure ≥130 mm Hg or diastolic blood pressure ≥85 mm Hg or treatment for previously diagnosed arterial hypertension; and fasting glucose ≥100 mg/dL or type 2 DM previously diagnosed. Laboratory data were obtained after 12-h overnight fasting and included hemoglobin, platelets, fasting glucose, insulin, urea, serum creatinine (Cr), uric acid, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, tri-

glycerides, total bilirubin, direct bilirubin, gamma-glutamyltransferase, alkaline phosphatase, alanine aminotransferase; aspartate aminotransferase, lactate dehydrogenase, albumin, total proteins, serum iron, total iron-binding capacity, ferritin and transferrin saturation, C-reactive protein, prothrombin time, international normalized ratio, and partial thromboplastin time. The Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) [28] that includes fasting insulin and glucose was calculated. Urinary Cr and albumin were obtained from a morning spot urine sample. EKD was defined as presence of microalbuminuria (urinary albumin-Cr ratio 30–300 mg/g [3–30 mg/mmol]) in a morning spot urine sample and estimated glomerular filtration rate (eGFR) ≥60 mL/min/1.73 m^2, calculated using the formula Chronic Kidney Disease Epidemiology Collaboration, that includes gender, race, and serum Cr [29].

Transient Elastography

TE was performed using a dedicated device (Fibroscan® Compact 530; Echosens, Paris, France) in patients with a minimum fasting of 2 h [30, 31]. Measurements were performed using the M or XL probe as appropriate on the right lobe of the liver through 9–11th intercostal space on the middle axillary line with the patient lying in a dorsal position with the right arm in maximal abduction, by an experienced operator that had undergone formal training and performed at least 500 examinations. The variables collected were LSM (kPa), controlled attenuation parameter (CAP) (dB/m), the type of probe used (M or XL), and interquartile variability (IQR) for LSM (%). The criteria for considering valid measurement was the presence of 10 valid measurements and IQR for LSM ≤30% or LSM <7.1 kPa when the IQR was >30% [32].

Significant liver fibrosis was defined as LSM ≥8.2 kPa with M or XL probe [23]. Liver steatosis was defined as CAP ≥250 dB/m with M probe of the Fibroscan® [22].

The maximum time interval between the scheduled evaluation, laboratorial analysis, and TE performance was 3 months.

Statistical Analysis

Data analysis of the results was performed using the Statistical Package for the Social Sciences (SPSS) software, version 26.0 (IBM, Armonk, NY, USA) and MedCalc® version 19.6 (MedCalc Software, Seoul, Republic of Korea). Continuous variables were expressed as means ± standard deviations. Categorical variables were expressed as absolute frequency (number) and relative frequency (percentages). Univariate analysis was performed by using χ^2 test or Fisher's exact test (2-tailed) as appropriate for categorical variables and by using Student's *t* test for independent samples for continuous normal-distributed variables. A multivariate analysis to identify potential independent predictive factors of EKD, adjusting for potential confounders, was performed by operating a binary logistic regression using the enter method. The selection of potential confounders to include in the multivariate analysis was performed by considering parameters with statistical significance in the univariate analysis and parameters described in the literature as being associated with kidney dysfunction. The discriminative capacity of LSM values to predict the presence of EKD was evaluated by the area under the receiver operating characteristic curve with 95% confidence interval (CI). The optimal cutoff value of LSM predictive of the presence of EKD was identified with the Youden Index, assessed by MedCalc® software. A *p* value of 0.05 was considered statistically significant.

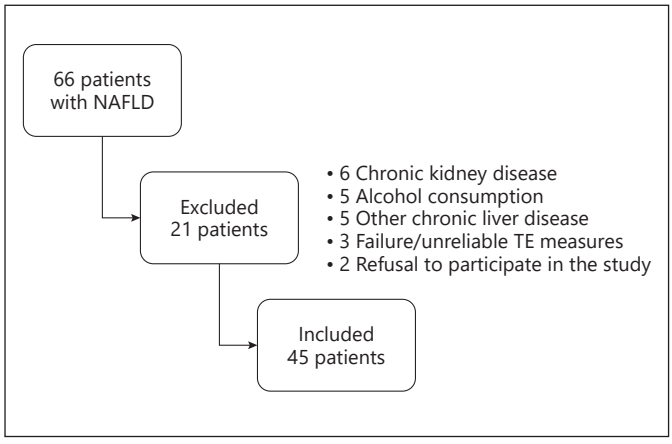

Fig. 1. Sampling process chart with the selection of patients in the study cohort. MAFLD, metabolic-associated fatty liver disease; TE, transient elastography.

Results

Characterization of Patients and Transient Elastography Parameters

We prospectively analyzed, during the study period, 66 patients with MAFLD scheduled for evaluation in a gastroenterology and hepatology consultation. After application of the defined exclusion criteria were eligible 45 patients with MAFLD. The sampling process is represented in Figure 1. A diagnostic biopsy of MAFLD was present in 22.2% of the patients. The detailed patient characteristics are described in Table 1. Of the 45 patients included, 53.3% were female gender; all were of Caucasian race with mean age of 53.5 ± 10.9 years. A minority of patients were smokers (6.7%), although a considerable percentage had comorbidities such as arterial hypertension (46.7%), type 2 DM (24.4%), dyslipidemia (60.0%), metabolic syndrome (46.7%), cardiovascular (4.4%) and cerebrovascular disease (2.2%), and hyperuricemia (2.2%). With regard to type 2 DM, all the patients were on oral antidiabetics and 2 were concomitantly on insulin. The patients had a mean HOMA-IR of 4.1 ± 3.2, reflecting insulin resistance. In the scheduled evaluation was verified that the patients had a mean BMI of 30.3 ± 5.6 kg/m^2, 40.0% were obese (BMI ≥30 kg/m^2), the mean waist circumference was 100.5 ± 10.6 cm, and the mean systolic and diastolic blood pressure was 138 ± 14 and 84 ± 10 mm Hg, respectively. Regarding laboratory data, the mean serum Cr was 0.80 ± 0.17 mg/dL, the mean eGFR was 95.0 ± 14.7 mL/min/1.73 m^2, and the mean urinary albumin-Cr ratio was 30.4 ± 60.8 mg/g. EKD was found in 17.8% of patients.

Table 1. Patient characteristics

	Value	Reference range
Demographic and anthropometric data		
Age, mean ± SD, years	53.5±10.9	
Female, *n* (%)	24 (53.3)	
Caucasian, *n* (%)	45 (100)	
Weight, mean ± SD, kg	80.2±13.9	
Height, mean ± SD, m	1.63±0.09	
BMI, mean ± SD, kg/m^2	30.3±5.6	
Waist circumference, mean ± SD, cm	100.5±10.6	
Obesity (BMI ≥30), *n* (%)	18 (40.0)	
Clinical data		
Systolic blood pressure, mean ± SD, mm Hg	138±14	
Diastolic blood pressure, mean ± SD, mm Hg	84±10	
Smoking, *n* (%)	3 (6.7)	
Arterial hypertension, *n* (%)	21 (46.7)	
Type 2 diabetes, *n* (%)	11 (24.4)	
Dyslipidemia, *n* (%)	27 (60.0)	
Metabolic syndrome, *n* (%)	21 (46.7)	
Hyperuricemia, *n* (%)	1 (2.2)	
Cerebrovascular disease, *n* (%)	1 (2.2)	
Cardiovascular disease, *n* (%)	2 (4.4)	
Insulin, *n* (%)	2 (4.4)	
Oral antidiabetics, *n* (%)	11 (24.4)	
Metformin, *n* (%)	10 (22.2)	
Statins, *n* (%)	23 (51.1)	
Fibrates, *n* (%)	8 (17.8)	
Laboratory data		
Hemoglobin, mean ± SD, g/dL	14.4±1.5	12.0–16.0
Platelets, mean ± SD, 10^9/L	231±44	150–350
Fasting glucose, mean ± SD, mg/dL	111±35	74–106
Insulin, mean ± SD, U/mL	14.6±7.7	2–25
Uric acid, mean ± SD, mg/dL	5.2±1.5	2.6–7.2
TG, mean ± SD, mg/dL	131±57	30–150
TC, mean ± SD, mg/dL	172±22	<200
HDL-c, mean ± SD, mg/dL	50±11	40–60
LDL-c, mean ± SD, mg/dL	96±23	<130
Urea, mean ± SD, mg/dL	38±10	15–39
SCr, mean ± SD, mg/dL	0.80±0.17	0.57–1.11
Ferritin, mean ± SD, ng/mL	290±236	8–252
Transferrin saturation, mean ± SD, %	28.0±60.6	15.0–50.0
Total bilirubin, mean ± SD, mg/dL	0.60±0.36	0.2–1.0
Direct bilirubin, mean ± SD, mg/dL	0.16±0.06	0.0–0.2
AST, mean ± SD, U/L	30±23	15–37
ALT, mean ± SD, U/L	49±28	30–65
G-GT, mean ± SD, U/L	96±160	5–85
ALP, mean ± SD, U/L	76±25	45–117
LDH, mean ± SD, U/L	180±35	84–246
Albumin, mean ± SD, g/dL	4.9±5.5	3.4–5.0
Total proteins, mean ± SD, g/dL	7.3±0.8	6.4–8.2
CRP, mean ± SD, mg/L	3.7±1.8	<3.0
aPTT, mean ± SD, s	31.2±2.7	20.9–34.9
PT, mean ± SD, s	11.7±0.95	8.4–14.4
INR, mean ± SD	1.03±0.09	
UCr, mean ± SD, mg/dL	109±56	20–300
UAlb, mean ± SD, mg/L	23±48	<20

Table 1 (continued)

	Value	Reference range
UAlb-UCr ratio, mean ± SD, mg/g	30.4±60.8	<30
eGFR, mean ± SD, mL/min/1.73 m^2	95.0±14.7	
EKD, *n* (%)	8 (17.8)	
HOMA-IR, mean ± SD	4.1±3.2	0.7–2.0
Transient elastography data		
M probe, *n* (%)	40 (88.9)	
LSM, mean ± SD, kPa	6.3±2.8	
CAP, mean ± SD, dB/m	295±70	
Liver fibrosis (LSM ≥8.2), *n* (%)	8 (17.8)	
Liver steatosis (CAP ≥250), *n* (%)	36 (80.0)	

SD, standard deviation; TG, triglycerides; TC, total cholesterol; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; SCr, serum Cr; AST, aspartate aminotransferase; ALT, alanine aminotransferase; G-GT, γ-glutamyl transferase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase; CRP, C-reactive protein; aPTT, partial thromboplastin time; PT, prothrombin time; INR, international normalized ratio; UCr, urinary Cr; UAlb, urinary albumin; eGFR, estimated glomerular filtration rate; EKD, early kidney dysfunction; HOMA-IR, Homeostasis Model Assessment of Insulin Resistance; LSM, liver stiffness measurement; CAP, controlled attenuation parameter.

The majority of the Fibroscan® examinations were performed with the M probe (88.9%), the mean LSM was 6.3 ± 2.8 kPa, and the mean CAP was 295 ± 70 dB/m. The presence of liver steatosis (CAP ≥250) was verified in 80.0% of patients, and 17.8% had significant liver fibrosis (LSM ≥8.2 kPa) (Table 1).

Predictors of EKD

In univariate analysis, we found that MAFLD patients with EKD were significantly more obese (75.0 vs. 32.4%, $p = 0.045$) and had significantly higher LSM (8.5 ± 4.1 vs. 5.8 ± 2.2 kPa, $p = 0.01$) and higher CAP levels in Fibroscan® (348 ± 44 vs. 283 ± 69 dB/m, $p = 0.02$) (Table 2). Although MAFLD patients with EKD had significantly higher CAP levels in Fibroscan®, the presence of liver steatosis (CAP ≥ 250) was not statistically significant ($p = 0.18$). There was no association between age ($p = 0.75$), gender ($p = 0.25$), systolic ($p = 0.15$) and diastolic ($p = 0.44$) blood pressure, smoking status ($p = 0.45$), comorbidities, laboratory parameters, HOMA-IR ($p = 0.24$), and the presence of EKD (Table 2).

The Role of TE in Predicting EKD

In multivariate analysis, after adjustment of potential confounders including age, obesity, arterial hypertension, and type 2 DM, the presence of significant liver fibrosis in TE remained a significant predictor of EKD, being associated with a 14.3-fold increased risk of EKD (OR: 14.3; 95% CI: 1.2–169.3; and $p = 0.04$) (Table 2). Obesity was also a significant predictor of EKD (OR: 14.1; 95% CI: 1.1–182.9; and $p = 0.04$) (Table 2).

LSM was found to accurately identify the presence of EKD (AUC 0.76; 95% CI: 0.60–0.87; and $p = 0.03$). The optimal cutoff value of LSM to predict EKD was 6.1 kPa with a sensitivity of 85.7% (95% CI: 42.1–99.6%) and a specificity of 67.6% (95% CI: 50.2–82.0%) (Fig. 2).

Discussion

In the present study, we found that approximately one fifth of the patients with MAFLD had microalbuminuria with eGFR ≥60 mL/min/1.73 m^2, defined as EKD. Previous evidence showed an increased risk of albuminuria among patients with MAFLD [33–37], even in those without DM [38]. It has been postulated that MAFLD could contribute to albuminuria by enhancing the endothelial dysfunction from the systemic inflammation [16, 39]. Microalbuminuria has been linked to early cardiovascular mortality [40, 41]. So, early detection and treatment of microalbuminuria to prevent cardiovascular and renal disease in patients with MAFLD is essential.

Many of the risk factors for MAFLD have the potential to influence the development of EKD. Obesity is a recognized direct and indirect risk factor for CKD and is strongly linked with MAFLD [42], so it was not surprising

Table 2. Predictors of early kidney dysfunction

	Univariate analysis			Multivariate analysis**		
	no EKD (n = 37)	EKD (n = 8)	p value	OR	95% CI	p value
Age, years	53.7±10.2	52.4±14.4	0.75	1.0	0.9–1.1	0.9
Female, %	48.6	75.0	0.25	–	–	–
Systolic pressure, mm Hg	137±12	145±21	0.15	–	–	–
Diastolic pressure, mm Hg	84±11	81±8	0.44	–	–	–
Smoking, %	5.4	12.5	0.45	–	–	–
Obesity, %	32.4	75.0	**0.045***	14.1	1.1–182.9	**0.04***
Arterial hypertension, %	45.9	50.0	1.0	0.24	0.02–3.4	0.29
Type 2 diabetes, %	18.9	50.0	0.09	3.6	0.27–47.2	0.34
Dyslipidemia, %	59.5	62.5	1.0	–	–	–
Metabolic syndrome, %	43.2	62.5	0.44	–	–	–
Hyperuricemia, %	2.7	0.0	1.0	–	–	–
Cerebrovascular disease, %	2.7	0.0	1.0	–	–	–
Cardiovascular disease, %	2.7	12.5	0.33	–	–	–
Insulin, %	2.7	12.5	0.33	–	–	–
Oral antidiabetics, %	18.9	50.0	0.09	–	–	–
Statins, %	54.1	37.5	0.46	–	–	–
Fibrates, %	18.9	12.5	1.0	–	–	–
Hemoglobin, g/dL	14.6±1.3	13.6±2.0	0.08	–	–	–
Platelets, 10^9/L	228±45	244±35	0.37	–	–	–
Fasting glucose, mg/dL	107±33	130±42	0.10	–	–	–
Insulin, U/mL	13.9±7.5	17.7±8.3	0.21	–	–	–
Uric acid, mg/dL	5.1±1.4	5.6±1.8	0.37	–	–	–
TG, mg/dL	127±50	148±86	0.36	–	–	–
TC, mg/dL	173±20	168±32	0.73	–	–	–
HDL-c, mg/dL	50±12	48±7	0.63	–	–	–
LDL-c, mg/dL	97±21	90±35	0.61	–	–	–
Urea, mg/dL	39.0±10.0	34.4±12.6	0.26	–	–	–
SCr, mg/dL	0.80±0.2	0.79±0.2	0.83	–	–	–
Ferritin, ng/mL	315±242	179±175	0.14	–	–	–
Transferrin saturation, %	29.3±62.7	22.4±38.8	0.30	–	–	–
Total bilirubin, mg/dL	0.64±0.4	0.43±0.2	0.16	–	–	–
Direct bilirubin, mg/dL	0.17±0.07	0.14±0.03	0.21	–	–	–
AST, U/L	27±18	40±38	0.15	–	–	–
ALT, U/L	45±23	64±44	0.10	–	–	–
G-GT, U/L	102±176	65±36	0.55	–	–	–
ALP, U/L	76±25	80±24	0.62	–	–	–
LDH, U/L	178±36	190±35	0.41	–	–	–
Albumin, g/dL	5.1±6.1	4.0±0.3	0.61	–	–	–
Total proteins, g/dL	7.2±0.8	7.6±0.3	0.16	–	–	–
CRP, mg/L	3.6±1.8	4.2±1.7	0.46	–	–	–
aPTT, s	30.9±2.5	32.6±3.3	0.10	–	–	–
PT, s	11.7±0.8	11.8±1.6	0.88	–	–	–
INR	1.0±0.1	1.0±0.2	0.95	–	–	–
HOMA-IR	3.9±3.4	5.4±2.2	0.24	–	–	–
LSM, kPa	5.8±2.2	8.5±4.1	**0.01***	–	–	–
CAP, dB/m	283±69	348±44	**0.02***	–	–	–
Liver fibrosis (LSM ≥8.2), %	10.8	50.0	**0.02***	14.3	1.2–169.3	**0.04***
Liver steatosis (CAP ≥250), %	75.7	100	0.18	–	–	–

EKD, early kidney dysfunction; TG, triglycerides; TC, total cholesterol; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; SCr, serum Cr; AST, aspartate aminotransferase; ALT, alanine aminotransferase; G-GT, γ-glutamyl transferase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase; CRP, C-reactive protein; aPTT, partial thromboplastin time; PT, prothrombin time; INR, international normalized ratio; HOMA-IR, Homeostasis Model Assessment of Insulin Resistance; LSM, liver stiffness measurement; CAP, controlled attenuation parameter; OR, odds ratio; CI, confidence interval. * Statistically significant (p < 0.05). ** Binary logistic regression.

that in our cohort obesity increased significantly the risk of EKD.

European guidelines for the management of MAFLD recommend that follow-up is mandatory in obesity since it is the major phenotype and risk condition for MAFLD, driven by insulin resistance, and also increases the risk of advanced disease [1]. So, we reinforce that monitoring of kidney function in obese patients with MAFLD should be encouraged.

Diabetes is also a recognized risk factor for CKD and is strongly related with MAFLD [18]. Previous studies indicated that MAFLD is not associated with decline in renal function in patients with type 2 DM [43] and that other factors such as blood pressure and plasma glucose might have more significant influence than MAFLD to increase the risk of albuminuria among these patients [38]. In our results, type 2 DM was tendential but not a significant predictor of EKD ($p = 0.09$). We think that this can be explained in part by the small sample size of the study and by the fact that our population is not very aged (mean age 53.5 ± 10.9), few are insulin dependent ($n = 2$), and the kidney damage may not yet be installed at the present time of analysis.

We did not find a significant association between liver steatosis and EKD in patients with MAFLD. This is in line with Yeung et al.'s [44] study that showed that advanced liver fibrosis but not steatosis was independently associated with albuminuria in patients with type 2 DM.

We found an association between significant liver fibrosis evaluated by TE and EKD. As age, arterial hypertension, obesity, and diabetes could be potential confounders for EKD, we included them in a multivariate analysis to adjust for potential confounders to control this bias, and after this adjustment, significant liver fibrosis persisted as a significant risk factor for EKD. This corroborates that patients with MAFLD with liver fibrosis in TE have a factual increased risk of EKD independently of established renal risk factors and potential confounders.

The area under the ROC curve of the LSM-EKD model for identifying EKD was 0.76 (95% CI: 0.60–0.87) with a reasonable discriminating ability (AUC ≥0.70). We also found that a cutoff value of LSM of 6.1 kPa was the most accurate to predict EKD (sensitivity: 85.7%; specificity: 67.6%). Although this LSM value corresponds to an initial stage of liver fibrosis, we accepted and recommend this value given its high sensitivity for the diagnosis of EKD. These findings expand other results recently published: Yeung et al. [45] demonstrated that advanced fibrosis assessed by TE was independently associated with an increased risk of albuminuria in a cohort of 1,763 Chinese

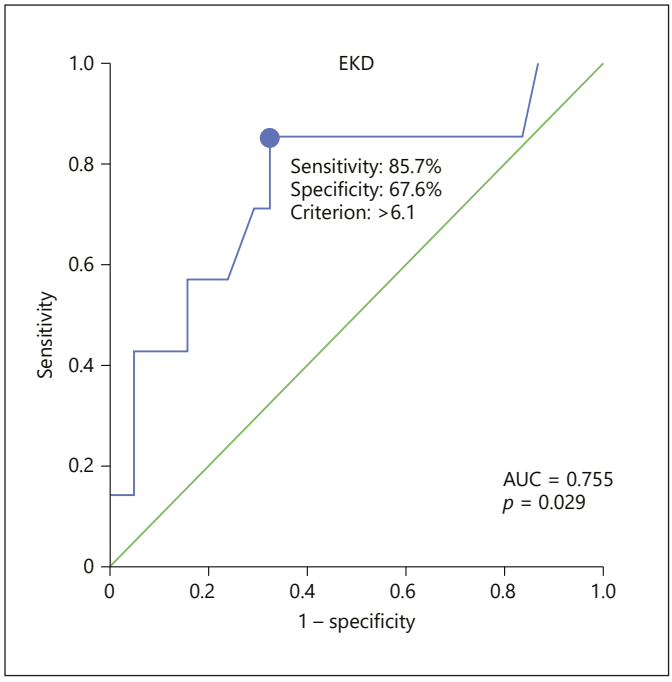

Fig. 2. LSM receiver operating characteristic curve with the optimal cutoff value for predicting EKD. LSM, liver stiffness measurement; EKD, early kidney disease.

patients with type 2 DM (adjusted OR: 1.52; $p = 0.039$). Qin et al. [24] reported that increased LSM evaluated by TE was a potential indicator of CKD in a cohort of 1,439 Chinese patients with ultrasound-detected MAFLD. More recently, Sun et al. [25] showed that LSM measured by TE can accurately identify those patients with MAFLD who are at risk of having EKD. Our results are clinically important because TE revealed to be an essential tool that allows not only to reliably stage liver fibrosis in MAFLD but also to predict MAFLD patients at increased risk of EKD. Importantly, CKD is associated with increased mortality in patients with MAFLD [8]. However, the European guidelines state that the optimal follow-up of patients with MAFLD is as yet undetermined [1] and there are no specific recommendations how to surveil renal function of these patients. On the other hand, improvement of MAFLD and metabolic syndrome is associated with a reduced incidence of type 2 DM and improved kidney function [5, 46, 47]. So, early detection of kidney dysfunction should be encouraged and be part of the routine management of patients with MAFLD in order to timely manage comorbidities and improve the patient's metabolic and cardiovascular profile to prevent progression to CKD. Based on our study and in those previously cited, we believe that patients with MAFLD should be

regularly monitored for renal function and for assessment of microalbuminuria, particularly if they have LSM >6.1 kPa.

TE is currently a widely available, noninvasive method used in clinical practice in many centers to accurately detect advanced liver fibrosis and cirrhosis in MAFLD patients [20–23]. Microalbuminuria, although a simpler and more available method, is generally not requested in clinical practice in MALFD patients unless there is already some degree of renal impairment documented by elevated serum Cr. So, currently when it is requested, patients have already a decrease in GFR or CKD, and this stage is ultimately what has to be avoided. Besides, urinary albumin-to-Cr ratio is affected by several factors such as age, gender, exercise, muscle mass, and others [48] and is important to ensure that the urine sample is the first-void spot urine specimen, as it is the preferred method for assessment of albuminuria [49] and random spot urine specimen appears to overestimate the prevalence of albuminuria [49, 50]. Thus, TE could help to make a preselection of MAFLD patients from whom we should actually request albuminuria to increase the accuracy of the analysis result. Therefore, we think that TE is very useful for EKD screening in patients with MAFLD because it is currently a widely available and noninvasive method that could enable in patients with LSM >6.1 kPa, as defined in our study, to request for microalbuminuria and thus to timely detect EKD and adopt measures to prevent the progression to CKD. Furthermore, it could eventually increase the patient's compliance to nonpharmacological and pharmacological measures knowing in advance that already have liver fibrosis and kidney dysfunction that worsen the prognosis.

Our study has some limitations. We did not use biopsy as a diagnostic method of MAFLD for all patients; only 22.2% of the patients were diagnosed by liver biopsy. Although liver biopsy is still the gold standard to diagnose MAFLD, it is limited by invasiveness, complications, expense, sampling error, and variability in interpretation which impairs its use in terms of diagnostic/screening method [1, 51–53]. Furthermore, European guidelines for the management of MAFLD recommend using ultrasonography as first-choice imaging in adults at risk for MAFLD because it is more widely available and cheaper than the gold standard, robustly diagnoses moderate and severe steatosis, and provides additional hepatobiliary information [1]. Moreover, there are other established risk factors for CKD that we did not include in the multivariate analysis as adiposity measures (BMI and waist circumference), and HOMA-IR score, in order to avoid multicollinearity between the variables since many of them were inherently correlated such as obesity and adiposity measures. Finally, a main shortcoming of TE is unreliable results in the presence of high BMI and/or thoracic fold thickness, as presented in a large, unselected European series, where up to 20% of examinations had unreliable results [54], mainly in obese MAFLD [55]. Although, this limitation was overcome by the use of XL probe, which has previously been shown to reduce the failure rate [56, 57]. Another limitation of the study is its cross-sectional design that does not allow to establish temporal and causal associations between liver fibrosis and presence of EKD. Furthermore, despite the significant association between significant liver fibrosis and risk of EKD ($p = 0.04$), the CI is large (95% CI: 1.2–169.3), likely due to the small sample size of the study, that is an important drawback of the study, justified by its prospective design. Moreover, the small sample size of the study may also have contributed to the fact that type 2 DM do not reach statistical significance as a predictor of EKD. However, despite this limitation, the prospective nature of the study is a strength, the association between significant liver fibrosis and risk of EKD was high (OR: 14.3), and our results are reproducible to those of Sun et al. [25], in a Western population, being valuable in improving decisions in the follow-up of the MAFLD patients.

To the best of our knowledge, only one study has yet assessed the role of TE in identifying the patients with MAFLD who are at risk of having EKD in clinical practice [25]. Therefore, further investigation is necessary to prove these results and establish a robust causal relationship between liver fibrosis and EKD, regardless of the associated comorbidities, in patients with MAFLD and to understand the evolution to ESRD and how to best manage these patients so as to halt this progression.

In conclusion, the present study revealed that liver fibrosis evaluated by a noninvasive method such as TE is associated with a significant increased risk of EKD in patients with MAFLD, independently of other comorbidities that are traditional renal risk factors, such as age, hypertension, and type 2 DM. Thus, TE could be useful as a screening method that alerts for timely identification of MAFLD patients at increased risk of EKD and associated comorbidities, stressing the need for in-depth hepatological investigation and intensive therapies and assisting in the referral of these patients to tertiary-care centers for more careful surveillance, to prevent the development of CKD in the long term and improve outcomes.

Statement of Ethics

The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution's human research committee (Ethics Committee of Hospital Senhora da Oliveira, Guimarães; approved September 10, 2019). All patients signed an informed consent form to participate in the study.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Freitas M. designed the study, acquisition and analysis of data, did the literature research, and drafted the manuscript. Magalhães J. designed the study, participated on the acquisition of data, performed the Fibroscan® examinations, and revised the manuscript. Macedo Silva V. and Xavier S. participated on the acquisition of data. Marinho C. participated on the acquisition of data and revised the manuscript. Cotter J. critically revised the manuscript and approved the final version to be submitted.

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