Digestive Diseases and Sciences
https://doi.org/10.1007/s10620-021-07363-x

ORIGINAL ARTICLE

Validation and Performance of FibroScan®-AST (FAST) Score on a Brazilian Population with Nonalcoholic Fatty Liver Disease

Ana Carolina Cardoso[1] · Cristiane Valle Tovo[2] · Nathalie Carvalho Leite[1] · Ibrahim A. El Bacha[3] · Fernanda Luiza Calçado[1] · Gabriela Perdomo Coral[2] · Glauco Navas Sammarco[3] · Claudia Cravo[1] · Roberto José Carvalho Filho[3] · Renata de Mello Perez[1] · Ronir Raggio Luiz[4] · Edison Roberto Parise[3] · Cristiane A. Villela-Nogueira[1]

Received: 18 July 2021 / Accepted: 7 December 2021

Abstract

Background and Aim FAST score has a good performance for diagnosing the composite of NASH + NAS ≥ 4 + F ≥ 2. However, it has not been evaluated in Latin American individuals with nonalcoholic fatty liver disease (NAFLD). We aimed to analyze the performance of the FAST score in a Brazilian NAFLD population.

Methods Cross-sectional study was held in ≥ 18 years NAFLD patients diagnosed by ultrasonography and submitted to liver biopsy (LB). Liver stiffness (LSM) and CAP measurements were performed with FibroScan®, using M (BMI < 32 kg/m^2) or XL probes. Area under receiver operating characteristic (AUROC) curves were calculated as well as sensitivity (S), specificity (Spe), positive predictive value (VPP) and negative predictive value (NPV) for the previously established FAST score cut-offs.

Results Among 287 patients included (75% female; mean age 55 ± 10 years), NASH + NAS ≥ 4 + F ≥ 2 was reported in 30% of LB. For the FAST cut-off of 0.35, the S and NPV to rule out NASH + NAS ≥ 4 + F ≥ 2 were 78.8% and 87.8%, respectively. Regarding the cut-off of 0.67, the Spe and PPV to rule-in NASH + NAS ≥ 4 + F ≥ 2 were 89.1%, 61.8%, respectively. The AUROC of FAST for all included patients was 0.78 (95% CI 0.72–0.84) and for those with ≥ 32 kg/m^2 was 0.81 (95% CI 0.74–0.88).

Conclusion FAST score has a good performance in a Brazilian NAFLD population, even in patients with higher BMI when the XL probe is adopted. Therefore, FAST can be used as a noninvasive screening tool mainly for excluding the diagnosis of progressive NASH, reducing the number of unnecessary liver biopsies.

Keywords FAST score · NAFLD · NASH · FibroScan® · Steatosis

Abbreviations

NAFLD	Nonalcoholic fatty liver disease
NASH	Steatohepatitis
TE	Transient elastography
CAP	Controlled Attenuation Parameter
LSM	Liver stiffness measurement
AST	Aspartate aminotransferase
FAST score	FibroScan®-AST
PPV	Positive predictive value
NPV	Negative predictive value
HIV	Human immunodeficiency virus
HCV	Hepatitis C virus
HBV	Hepatitis B virus
ICF	Informed consent form
BMI	Body mass index
ALT	Alanine aminotransferase

✉ Cristiane A. Villela-Nogueira
crisvillelanog@gmail.com

[1] Hepatology Unit - Clementino Fraga Filho University Hospital – School of Medicine, Federal University of Rio de Janeiro, Rua Professor Rodolpho Paulo Rocco 255 - Room 9E16, Rio de Janeiro 29913-941, Brazil

[2] Gastroenterology and Hepatology Unit, Department of Internal Medicine, Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA), Porto Alegre, Brazil

[3] Division of Gastroenterology, Hepatology Section, Federal University of São Paulo, São Paulo, Brazil

[4] Institute of Public Health Studies, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

Published online: 29 January 2022

GGT	Gammaglutamil transferase
kPa	Kilopascal
dB/m	Decibel/meter
OR	Odds ratios
AUROC	Area under receiver operating characteristic
T2DM	Type 2 diabetes
SAH	Systemic arterial hypertension
S	Sensitivity
Spe	Specificity

Introduction

Nonalcoholic fatty liver disease (NAFLD) is the most prevalent cause of chronic liver disease in the western countries [1, 2]. However, despite its rising prevalence, the best technique to evaluate the severity of the disease is a source of great debate. Liver biopsy is considered the gold standard for diagnosing and evaluating the histopathological features that define the presence of steatohepatitis, the progressive hallmark of NAFLD, characterized by the presence of steatosis, ballooning and lobular inflammation, with or without fibrosis [3]. Nevertheless, as extensively discussed in the literature, liver biopsy is considered an imperfect gold standard because it is an invasive procedure, with a small liver area assessed, sampling and interobserver variability, the need of expertise to perform and not negligible morbidity/mortality. Likewise, it is not useable for population screening [4, 5]. Although liver fibrosis is related to the prognosis and mortality of liver disease [6], the evaluation of steatohepatitis (NASH) is increasingly required because it encompasses a higher risk of progression, and it is still requested for the inclusion of patients in clinical trials [3, 7]. The development of new molecules for NAFLD treatment is currently the subject of several studies worldwide and the majority requires the diagnosis of NASH in the baseline [8, 9]. Transient elastography (TE) (FibroScan®, Echosens, Paris, France) has emerged as a popular noninvasive evaluation of liver fibrosis with optimal results reported for diagnosing cirrhosis and advanced fibrosis in NAFLD/NASH [10–12]. Although the high prevalence of NAFLD in obese patients [1, 13], TE showed lower performance in this specific population, which characterized a limitation of the method [14, 15]. However, the incorporation of the XL probe (with an increased skin-liver length) significantly modified this scenario [16–18]. Furthermore, the incorporation of Controlled Attenuation Parameter (CAP), which quantifies the degree of hepatic steatosis simultaneously to the liver stiffness measurement (LSM), added more information about those patients [19, 20]. Evidence of similar performance of both probes (M and XL) in NAFLD patients, including the diagnosis of significant fibrosis and steatosis, is available [21–23]. Nonetheless, the diagnosis of NASH through TE is not possible. In 2020, Newsome et al. proposed a new score to identify patients with NASH, NAFLD activity Score (NAS) ≥ 4 and significant fibrosis (stage 2 or higher [F ≥ 2]) [24]. This score included liver stiffness and CAP measurements by FibroScan® combined with aspartate aminotransferase (AST) and was designated FAST Score (FibroScan®-AST). In the original study, the authors evaluated an international cohort from Asia, North American and Europe. They suggested a dual cut-off approach: a rule-out cut-off of 0.35 based on a sensitivity of 0.90 or greater and a rule-in cut-off of 0.67 for the specificity of 0.90 or greater, leading to a positive predictive value (PPV) of 0.83 and a negative predictive value (NPV) of 0.85. Patients were considered in a gray zone between the two cut-offs. They concluded that the FAST score provides an efficient way to identify patients at risk of progressive NASH noninvasively and reduce unnecessary liver biopsy in patients unlikely to have significant disease. To date, FAST performance was not evaluated in cohorts from Latin America. This way, we aimed to validate and analyze the performance of the FAST score in a Brazilian NAFLD population since it may be a valuable tool for the identification of the composite of NASH and significant fibrosis in NAFLD patients.

Methods

Study Design and Patients

This cross-sectional study with prospective inclusion was conducted at the outpatient unit of three references Hepatology Services in Brazil. Two Services are in university hospitals in the Southeast (Clementino Fraga Filho University Hospital, Federal University of Rio de Janeiro and São Paulo University Hospital, Federal University of São Paulo) and one in Southern Brazil (Santa Casa da Misericórdia de Porto Alegre University Hospital, Universidade Federal de Ciências da Saúde de Porto Alegre—UFCSPA). Individuals 18 years or older and NAFLD diagnosis by ultrasonography, whose clinicians requested a liver biopsy, based on an elevated liver stiffness (higher than 7.0 kPa) and/or abnormal aminotransferases were included in this study. HIV, HCV and HBV infected patients were excluded as those with other chronic liver diseases. Patients using hepatotoxic drugs or any therapy related to hepatic steatosis were excluded. Individuals with a daily alcohol intake of more than 20 g for women and 30 g for men were also excluded. In addition, subjects with a contraindication for liver biopsy were also excluded. Following the Declaration of Helsinki, the local Ethics Committee approved the study, and all patients signed an informed consent form (ICF).

Study Procedures

Individuals included in the study were submitted to clinical and laboratory evaluation, liver stiffness and CAP measurements using FibroScan® and liver biopsy with a maximum interval between all the procedures of 2 weeks.

Demographic, Clinical, and Laboratory Variables

Demographic (gender, age), anthropometric (body mass index—BMI, weight), clinical (diagnosis of type 2 diabetes, systemic arterial hypertension and dyslipidemia) and laboratory variables (alanine aminotransferase test—ALT, aspartate aminotransferase test—AST) were registered.

Liver Stiffness and CAP Measures

Liver stiffness and CAP measurements were performed with FibroScan® 502 touch (Echosens, Paris, France), using M or XL probes according to BMI (M probe was used in patients with BMI < 32 kg/m^2), as proposed in the reference study [24]. Patients were fast for at least 3 h before the examination. The FibroScan® technique was previously described [25, 26]. The final median is expressed in kilopascal (kPa). Only ten valid measurements, IQR/median liver stiffness ratio < 30% and success rate > 60%, were included in the analysis. CAP, an evaluation of the LSM attenuation to determine the amount of steatosis, was reported in decibel/meter (dB/m) [19]. Thus, both liver stiffness and CAP were obtained simultaneously and in the same volume of liver parenchyma. FibroScan® were realized by a physician masked to patients' clinical and liver biopsy data.

Liver Histopathology

Percutaneous liver biopsy was performed guided by ultrasound, with 16-gauge needles. Experienced physicians obtained the fragments according to standard procedures [5]. A single pathologist in each of the different centers, blinded to the study data, evaluated liver biopsy specimens. The NASH CRN Scoring system was applied to define steatosis, the presence of ballooning, lobular inflammation grades, NASH, NAS and fibrosis stage [3]. All included samples had at least 15 mm of length and at least 10 portal tracts. Those with less than these parameters were excluded.

FAST Score

Each center prepared a database with AST results, LSM and CAP using FibroScan®. All patients' identifications were entered anonymously and received a code. The databases were unified and sent to Echosens for the calculation of the FAST score. The FAST score was calculated using the following equation:

$$\mathrm{FAST} = \frac{e^{-1.65+1.07\times \mathrm{In(LSM)}+2.66*10^{-8}\times \mathrm{CAP3}-63.3\times \mathrm{AST}^{-1}}}{1+e^{-1.65+1.07\times \mathrm{In(LSM)}+2.66*10^{-8}\times \mathrm{CAP3}-63.3\times \mathrm{AST}^{-1}}}$$

Statistical Analysis

Clinical and laboratory data and liver stiffness and CAP final values and histopathology diagnosis were recorded in forms and entered in the SPSS© 24.0 software (IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.). All variables' values were analyzed as continuous variables or were categorized when appropriate. Variables with normal distribution were expressed by mean and standard deviation, and nonparametric variables were expressed as a median and interquartile interval. The primary analysis of this study was the evaluation of the diagnostic performance of the FAST score. Areas under receiver operating characteristic (AUROC) curves were calculated. In addition, sensitivity, Specificity, Positive Predictive Value and Negative Predictive Value for the previously described cut-offs were estimated in the included population.

Results

Study Population

Two hundred eighty-seven patients have agreed to participate in the study and have signed the ICF. All patients were submitted to laboratory evaluation, TE through FibroScan® with M or XL probes, according to BMI, and liver biopsy with a maximum delay of two weeks between the procedures. Demographic, clinical, and laboratory characteristics of the 287 patients included in the study are shown in Table 1. Most patients were female (75%) with a mean age of 55.1 ± 10.1 years. The mean BMI was 32.2 ± 5.4 kg/m^2. Only 6.5% of patients had a BMI under 25, and 63.9% of patients were obese. Type 2 Diabetes Mellitus (T2DM) was present in 56.9% and systemic arterial hypertension (SAH) in 63.6%. The included patients in the three different centers had similar characteristics considering age, BMI, the incidence of T2DM and aminotransferases levels (Table 1). However, the incidence of SAH and dyslipidemia was higher in Rio de Janeiro, as shown in Table 1.

Histological Characteristics

The distribution of steatosis and fibrosis according to the histological analysis is described in Table 1. The

Table 1 Clinical, demographic and laboratorial characteristics of all patients and according to each participant center ($n=287$)

Variables	All patients ($n=287$)	Rio de Janeiro ($n=115$)	São Paulo ($n=137$)	Porto Alegre ($n=35$)
Female—Gender (%)	75.3	78.3	72.9	72.0
Age (years)	55.110.1	54.010.1	55.510.1	59.29.7
BMI (kg/m^2)	32.25.4	33.66.0	30.94.3	31.65.9
BMI ≥ 32 (kg/m^2) (%)	47.0	62.5	34.8	26.0
BMI ≥ 40 (kg/m^2) (%)	9.7	15.7	0.0	12.5
Diabetes (%)	56.9	59.2	55.6	52.0
SAH (%)	63.6	81.1	51.9	40.0
Dyslipidemia (%)	69.9	82.6	62.4	48.0
AST (U/L)	30 (22–45)	28.5 (22–43.2)	30.5 (22–48.7)	32 (16–103)
ALT (U/L)	43.5 (29–64.2)	41.5 (30–65.2)	43.5 (29–62.5)	40.5 (29–63.1)
E (kPa) M probe	7.4 (5.7–11.7)	8.0 (6.1–11.9)	7.2 (3.0–65)	11.4 (5.4–26.3)
CAP (dB/m) M probe	311 (278–340)	313 (243–343)	303 (233–350)	321 (285–338)
E (kPa) XL probe	7.6 (4.7–13.0)	6.8 (4.9–10.1)	6.7 (5.4–9.7)	9.3 (3.5–22.4)
CAP (dB/m) XL probe	318 (288–336)	327 (291–359)	310 (273–339)	319 (280–305)
Steatosis (S)				
5% (%) [S0]	2.7	2.8	0	2.7
5–33% (%) [S1]	31.3	30.3	50	30.6
> 33–66% (%) [S2]	47.3	48.6	0	47.7
> 66% (%) [S3]	18.7	16.3	50	18.9
NAS ≥ 4 (%)	75.2	57.5	92.5	72.2
NAS	5.0 (3.0–6.0)	4.0 (2.0–5.0)	5.0 (4.0–6.0)	5.0 (3.0–6.0)
NASH (%)	80.4	68.5	88.7	96.0
NAS ≥ 4 + NASH + F ≥ 2 (%)	30	20.9	37.6	36.0
Fibrosis (F) Stage				
F0 (%)	26.4	34.6	21.2	12.0
F1 (%)	38.7	10.9	40.2	20.0
F2 (%)	12.7	9.4	15.2	16.0
F3 (%)	12.0	5.5	16.7	20.0
F4 (%)	10.2	9.4	6.8	32.0
F2–F4 (%)	34.6	24.6	39.1	68.0
FAST-score	0.32 (0.15–0.61)	0.32 (0.15–0.62)	0.29 (0.12–0.54)	0.50 (0.24–0.79)
≤ 0.35 (%)	51.7	51.2	56.1	32
0.351–0.669 (%)	28.3	28.7	26.5	36

Values are mean (standard deviation) for normally distributed data or median (interquartile interval) for nonparametric data. Categorical data are expressed by proportion. BMI, body mass index; SAH, systemic arterial hypertension; AST, aspartate aminotransferase; ALT, alanine aminotransferase; E, elastography, CAP, Controlled Attenuation Parameter

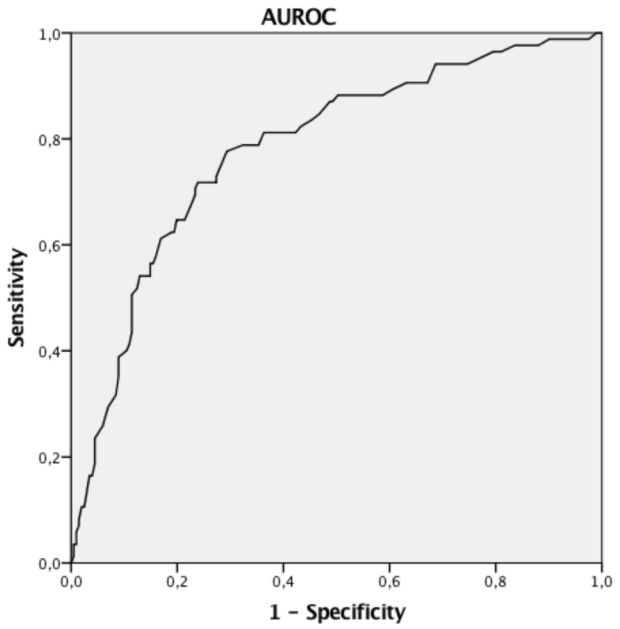
diagnosis of NASH was present in 80.4%, ballooning in 65.8% and NAS ≥ 4 in 75.2% of 287 patients. The presence of NAS ≥ 4 + NASH + F ≥ 2 was identified in 30% of the studied population (Table 1). The prevalence of each parameter—NASH, NAS > 4 and significant fibrosis—was different in the three centers, as present: Rio Grande do Sul (96%, 72.2% and 68%); São Paulo (88.7%, 92.5% and 39.1%) and Rio de Janeiro (68.5%, 57.5% and 24.6%), respectively. The finding of NAS ≥ 4 + NASH + F ≥ 2 was higher in the Rio Grande do Sul and São Paulo than in Rio de Janeiro individuals (Table 1).

Analysis of the Transient Elastography with the M and XL Probes

The results of the median LSM and the mean CAP with FibroScan® of the included patients are shown in Table 1. The XL probe was used in 47% of the included patients.

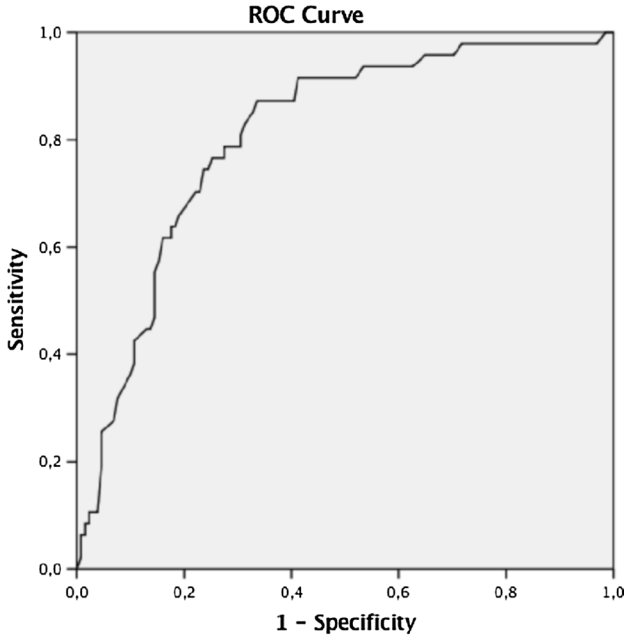
FAST Score

As previously described, 30% of the 287 included patients had NAS ≥ 4 + NASH + F ≥ 2 in the liver biopsies. The



FAST	
AUROC	0.78 (0.72-0.84)

Fig. 1 AUROC of FAST score in the general population



Diagonal segments are produced by ties.

FAST	
AUROC	0.81 (0.74-0.88)

Fig. 2 Area under the ROC curve of FAST score in patients with BMI ≥ 32 kg/m^2

prevalence of this combined histological results in the studied populations from Rio de Janeiro, São Paulo, and Porto Alegre is in Table 1. The median FAST result was 0.32 (0.01–0.94), and the majority (51.7%) of the individuals had a FAST ≤ of 0.35 (Table1). According to the population included in the present study, adopting the previously described cut-off of 0.35, the sensitivity (S), specificity (Spe), PPV and NPV to rule-out NAS ≥ 4 + NASH + F ≥ 2 were 78.8%, 64%, 48.6% and 87.8%, respectively. Regarding the cutoff of 0.67, the S, Spe, PPV and NPV to rule-in NAS ≥ 4 + NASH + F ≥ 2 were 41.2%, 89.1%, 61.8% and 77.9%, respectively. The AUROC of FAST score considering all included patients was 0.78 (95% CI 0.72–0.84) (Fig. 1). When only patients with BMI equal to or higher than 32 kg/m^2 (47%) were evaluated, the AUROC was 0.81 (95% CI 0.74–0.88) (Fig. 2). Figure 3 shows the sensitivity, specificity, PPV and NPV of FAST score according to the prevalence of the outcome variables (NAS ≥ 4 + NASH + F ≥ 2).

Discussion

This prospective multicenter study evaluates the performance of FAST score in a Brazilian population with NAFLD, which is predominantly obese. This study is the first that evaluates the performance of this new score that aims to identify patients with progressive NASH in a South American cohort. In our study, the diagnostic performance of FAST to rule out patients with NAS ≥ 4 + NASH + F ≥ 2, using the 0.35 cut-off, showed good sensitivity (78.8%) and high NPV (87.8%). Compared with the study of Newsome et al., the sensitivity was lower than those verified in most cohorts evaluated. On the other hand, the NPV, as in the included populations in the validation cohort, showed a high accuracy to exclude this progressive form of the disease [24]. To rule-in, the cut-off of 0.67 was associated with high specificity (89.1%) and a PPV of 61.8%, which was lower than those presented in Newsome paper's cohorts. However, the specificity was higher (89.1%) than most of the validation's cohorts [24]. The overall performance of FAST score, evaluated using the AUROC, was 0.78, and in obese individuals, most of the study population was 0.81. The evaluation of a South American population is important due to the high incidence of NAFLD in this geographic region (31%) [2, 7, 27], which was not assessed in the study that first reported the FAST score [24]. Ethnicity is an important predictor for chronic liver disease complications and has a strong influence on the response to treatment of chronic liver disease [28]. The population included in this

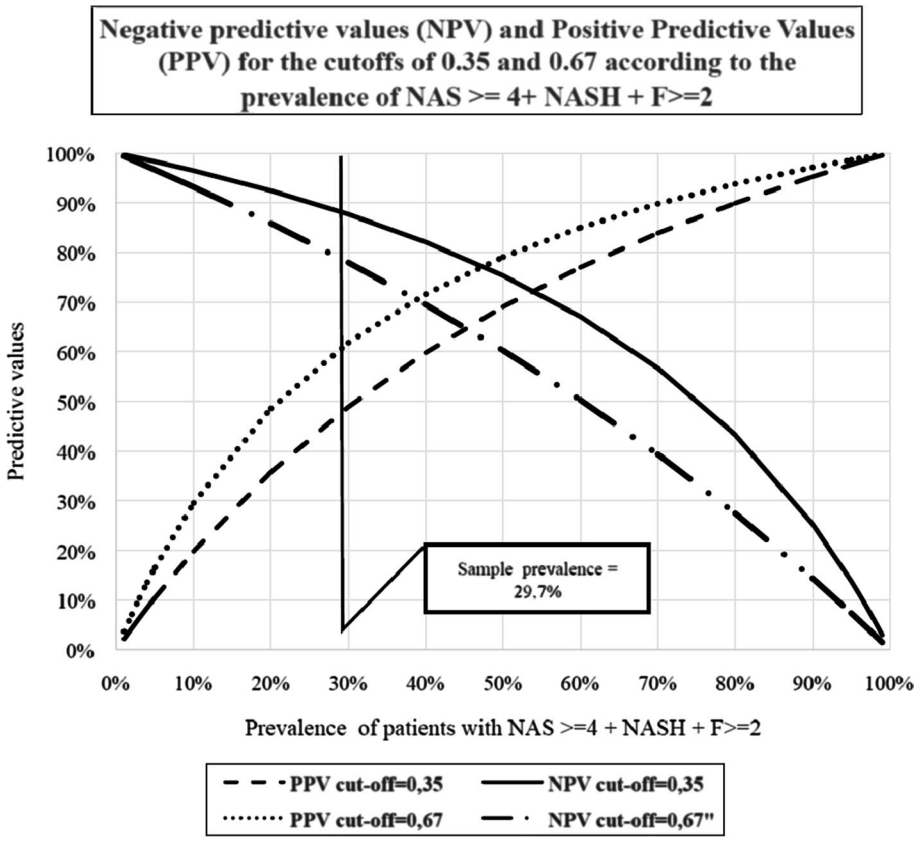


Fig. 3 Sensitivity, specificity, PPV and NPV of FAST score according to the prevalence of the outcome variables (NAS ≥ 4 + NASH + F ≥ 2)

study is composed of Brazilians, which are predominantly descendants of non-Hispanic groups, but from Amerindian, Afro descendants and Europeans (mostly Portuguese) and the mixture of these races [29, 30]. A multicentric study in Brazilian patients with NAFLD by Cotrim et al. [31] evaluated 1287 Brazilian NAFLD patients, of whom 487 had histological evaluation. A high prevalence of NASH among this group (58%) and also of NASH with liver fibrosis (27%) was diagnosed, showing that the prevalence of severe forms of NASH is considerably high in Brazil. Hence, FAST score may be a valuable tool to investigate the spectra of NAFLD in this population and the validation of the predefined cut-off points by Newsome et al. is needed. Our study evaluated individuals from the two most populated regions of Brazil, Southeast and South, which allows the analysis of a representative sample of our population. Therefore, as previously discussed, it is one of the regions with the highest worldwide prevalence of NAFLD. This way, to assess the incidence of a progressive form of NASH seems to be mandatory to stratify and reduce risks in the affected individuals. NASH seems to be a more aggressive form of the disease that progresses more commonly to advanced fibrosis and cirrhosis [32]. Therefore, the use of FAST score in this group can benefit the health system costs, excluding individuals with a lower risk of progression. In obese patients, NAFLD can be identified in a prevalence of up to 90% and 65% of overweight patients [33]. In the present study, only 6.5% of our patients had a BMI < 25 kg/m^2, 29.6% were overweight, and 63.9% were obese. The mean BMI in the derivation cohort and four of the validation cohorts (USA, French NAFLD, Turkish and in the pooled external patients' cohort) in the study by Newsome and collaborators [24] was similar to that found in our study. The Japanese population studied by Oeda et al. with FAST score had a lower median BMI in both verification and validation set (26.9 and 27.1 kg/m^2) [34], respectively. Despite similar weights in several analyzed cohorts, the presence of NASH was different in the studied populations, varying from 28% in the French bariatric surgery cohort (with higher BMI) to 96% in our population in southern Brazil. It is remarkable that, even with the differences, FAST score demonstrated similar performance in the studies. In Oeda study [34], the authors evaluated the accuracy of FAST score in 84 individuals with NAFLD and compared the performance between the FibroScan® M and XL probes. No significant differences were verified in FAST score between the M and XL probes, and no significant difference occurred in the AUROC between the two probes. In our study, we did not compare the performance of both probes, but the accuracy of the XL probe in patients with BMI ≥ 32 kg/m^2 was good. The AUROC of FAST in patients with BMI ≥ 32 kg/m^2 was 0.81. This result could be explained by the higher prevalence of individuals with the progressive form of

NASH in this group of Brazilian individuals. This study has some limitations. One of the limitations is using the cut-offs previously proposed by the original article and not assessing the best cut-off points for our studied population since it might result in better sensitivity, specificity, PPV and NPV. However, it is crucial to standardize an established cut-off to rule out and rule in NASH worldwide. The populations included in the three different centers were also different in frequency and severity of the liver disease, making standardization more complex and less accurate. However, it is a real-life cohort of NAFLD patients that attend liver clinics in university hospitals in Brazil. FAST score uses a specific device as FibroScan®, which might limit general applicability, especially in locations farther from the central reference services and large cities. However, regarding the diagnosis of NASH with significant fibrosis, it can be considered a valuable tool for daily use as a second step noninvasive tool, when Fibroscan® is available and a point-to-care equipment [35]. In conclusion, FAST score has a good performance in a Brazilian NAFLD population, similar to that obtained by Newsome et al. It is also adequate for screening NAFLD individuals with BMI higher than 32 mg/kg^2 if XL probe is adopted. Furthermore, this score can be used as a noninvasive screening tool, mainly for excluding the diagnosis of NASH and significant fibrosis in NAFLD patients, thus saving patients from a futile liver biopsy.

Author's contribution Cristiane A. Villela-Nogueira and Ana Carolina Cardoso contributed to the study conception and design. Material preparation and data collection were performed by Ana Carolina Cardoso, Cristiane Valle Tovo, Nathalie Carvalho Leite, Ibrahim A El Bacha, Fernanda Luiza Calçado, Gabriela Perdomo Coral, Glauco Navas Sammarco, Claudia Cravo, Roberto José Carvalho Filho, Renata de Mello Perez, Edison Roberto Parise and Cristiane A. Villela-Nogueira. Statistical analysis was performed by Ronir Raggio Luiz and Cristiane A. Villela-Nogueira. The first draft of the manuscript was written by Ana Carolina Cardoso, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding This work was supported by funding from Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ), Rio de Janeiro, and Conselho Nacional de Desenvolvimento Científico e Tecnológico, (CNPq), Brazil.

Declarations

Conflict of interest The authors have nothing to disclose.

Ethics approval All procedures performed in this study were following the ethical standards of the institutional and national research committee and with the 1964 Declaration of Helsinki and its later amendments. The local Ethics Committee (Hospital Universitário Clementino Fraga Filho) approved the study (CAAE No. 38752414.7.0000.5257).

Consent to participate Informed consent was obtained from all individual participants included in the study.

References

1. Williams CD, Stengel J, Asike MI et al. Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: a prospective study. *Gastroenterology.* 2011;140:124–131.
2. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology.* 2016;64:73–84.
3. Kleiner DE, Brunt EM, Natta MV et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology.* 2005;41:1313–1321.
4. Sumida Y, Nakajima A, Itoh Y. Limitations of liver biopsy and non-invasive diagnostic tests for the diagnosis of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. *World J Gastroenterol.* 2014;20:475–485. https://doi.org/10.3748/wjg.v20.i2.475.
5. Neuberger J, Patel J, Caldwell H et al. Guidelines on the use of liver biopsy in clinical practice from the British Society of Gastroenterology, the Royal College of Radiologists and the Royal College of Pathology. *Gut.* 2020;69:1382–1403. https://doi.org/10.1136/gutjnl-2020-321299.
6. Ekstedt M, Hagstrom H, Nasr P et al. Fibrosis stage is the strongest predictor for disease-specific mortality in NAFLD after up to 33 years of follow-up. *Hepatology.* 2015;61:1547–1554.
7. Sanyal AJ. Past, present and future perspectives in nonalcoholic fatty liver disease. *Nat Rev Gastroenterol Hepatol* 2019;16:377–386.
8. Younossi ZM, Loomba R, Rinella ME et al. Current and future therapeutic regimens for nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. *Hepatology.* 2018;68:361–371. https://doi.org/10.1002/hep.29724.
9. Cardoso AC, de Figueiredo-Mendes C, Villela-Nogueira CA, Sanyal AJ. New drugs for non-alcoholic steatohepatitis. *Liver Int.* 2020;40:96–101. https://doi.org/10.1111/liv.14354.
10. Wong GL, Wong VW-S, Choi PC-L et al. Assessment of fibrosis by transient elastography compared with liver biopsy and morphometry in chronic liver diseases. *Clin Gastroenterol Hepatol* 2008;6:1027–35.
11. Yoneda M, Yoneda M, Mawatari 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.
12. Wong VW, Vergniol J, Wong GL-H et al. Diagnosis of fibrosis and cirrhosis using liver stiffness measurement in nonalcoholic fatty liver disease. *Hepatology* 2010;51:454–462.
13. Bellentani S, Saccoccio G, Masutti F et al. Prevalence of and risk factors for hepatic steatosis in Northern Italy. *Ann Intern Med* 2000;132:112–117.
14. Castera L, Foucher J, Bernard P-H et al. Pitfalls of liver stiffness measurement: a 5-year prospective study of 13,369 examinations. *Hepatology* 2010;51:828–835.
15. Wong GL-H, Wong VW-S, Chim AM-L et al. Factors associated with unreliable liver stiffness measurement and its failure with transient elastography in the Chinese population. *J Gastroenterol Hepatol* 2011;26:300–5.
16. de Lédinghen V, Vergniol J, Foucher J, El-Hajbi F, Merrouche W, Rigalleau V. Feasibility of liver transient elastography with FibroScan using a new probe for obese patients. *Liver Int.* 2010;30:1043–1048.
17. Friedrich-Rust M, Hadji-Hosseini H, Kriener S et al. Transient elastography with a new probe for obese patients for noninvasive staging of non-alcoholic steatohepatitis. *Eur Radiol.* 2010;20:2390–2396.

18. Wong VW-S, Vergniol J, Wong GL-H et al. Liver stiffness measurement using XL probe in patients with nonalcoholic fatty liver disease. *Am J Gastroenterol.* 2012;107:1862–71.
19. Sasso M, Beaugrand M, de Ledinghen V et al. Controlled attenuation parameter (CAP): a novel VCTE guided ultrasonic attenuation measurement for the evaluation of hepatic steatosis: preliminary study and validation in a cohort of patients with chronic liver disease from various causes. *Ultrasound Med Biol.* 2010;36:1825–1835.
20. de Lédinghen V, Vergniol J, Foucher J, Merrouche W, le Bail B. Non-invasive diagnosis of liver steatosis using controlled attenuation parameter (CAP) and transient elastography. *Liver Int.* 2012;32:911–918.
21. de Lédinghen V, Wong VW-S, Vergniol J et al. Diagnosis of liver fibrosis and cirrhosis using liver stiffness measurement: comparison between M and XL probe of FibroScan. *J Hepatol* 2012;56:833–9.
22. de Lédinghen V, Hiriart JB, Vergniol J, Merrouche W, Bedossa P, Paradis V. Controlled attenuation parameter (CAP) with the XL probe of the Fibroscan®: a comparative study with the M probe and liver biopsy. *Dig Dis Sci* 2017;62:2569–2577. https://doi.org/10.1007/s10620-017-4638-3.
23. Cardoso AC, Cravo C, Calçado FL et al. The performance of M and XL probes of FibroScan for the diagnosis of steatosis and fibrosis on a Brazilian nonalcoholic fatty liver disease cohort. *Eur J Gastroentrol Hepatol.* 2020;32:231–238. https://doi.org/10.1097/MEG.0000000000001496.
24. Newsome PN, Sasso M, Deeks JJ et al. FibroScan-AST (FAST) score for the non-invasive identification of patients with non-alcoholic steatohepatitis with significant activity and fibrosis: a prospective derivation and global validation study. *Lancet Gastroenterol Hepatol.* 2020;5:362–373. https://doi.org/10.1016/S2468-1253(19)30383-8.
25. Sandrin L, Fourquet B, Hasquenoph J-M et al. Transient elastography: a new noninvasive method for assessment of hepatic fibrosis. *Ultrasound Med Biol* 2003;29:1705–1713.
26. de Lédinghen V, Vergniol J. Transient elastography (FibroScan). *Gastroenterol Clin Biol.* 2008;32:58–67.
27. Idalsoaga F, Kulkarni AV, Mousa OY, Arrese M, Arab JP. Non-alcoholic fatty liver disease and alcohol-related liver disease: two intertwined entities. *Front Med (Lausanne)* 2020;7:448. https://doi.org/10.3389/fmed.2020.00448.
28. Weston SR, Leyden W, Murphy R et al. Racial and ethnic distribution of nonalcoholic fatty liver in persons with newly diagnosed chronic liver disease. *Hepatology* 2005;41:372–379. https://doi.org/10.1002/hep.20554.
29. Parra FC, Amado RC, Lambertucci JR, Rocha J, Antunes CM, Pena SDJ. Color and genomic ancestry in Brazilians. *Proc Natl Acad Sci USA* 2003;100:177–182. https://doi.org/10.1073/pnas.0126614100.
30. Alves-Silva J, Da Silva Santos M, Guimarães PE. The ancestry of Brazilian mtDNA lineages. *Am J Hum Genet.* 2000;67:444–461. https://doi.org/10.1086/303004.
31. Cotrim HP, Parise ER, Oliveira CPMS et al. Nonalcoholic fatty liver disease in Brazil. Clinical and histological profile. *Ann Hepatol.* 2011;10:33–37.
32. Cotter TG, Rinella M. Nonalcoholic fatty liver disease 2020: the state of the disease. *Gastroenterology.* 2020;158:1851–1864. https://doi.org/10.1053/j.gastro.2020.01.052.
33. Argo CK, Caldwell SH. Epidemiology and natural history of non-alcoholic steatohepatitis. *Clin Liver Dis.* 2009;13:511–531. https://doi.org/10.1016/j.cld.2009.07.005.
34. Oeda S, Takahashi H, Imajo K et al. Diagnostic accuracy of FibroScan-AST score to identify non-alcoholic steatohepatitis with significant activity and fibrosis in Japanese patients with non-alcoholic fatty liver disease: comparison between M and XL probes. *Hepatol Res.* 2020;50:831–839. https://doi.org/10.1111/hepr.13508.
35. Castera L, Friedrich-Rust M, Loomba R. Noninvasive assessment of liver disease in patients with nonalcoholic fatty liver disease. *Gastroenterology.* 2019;156:1264-1281.e4. https://doi.org/10.1053/j.gastro.2018.12.036.