




# Fibrotic NASH in Individuals with Obesity: a Cross-sectional Analysis of the Prevalence of this Significant Milestone of Disease Progression and Accuracy of a Non-invasive Marker for its Screening

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## Abstract

**Background** Fibrotic non-alcoholic steatohepatitis (NASH), i.e., the concomitant presence of active inflammation and fibrosis, represents a milestone in the natural history of NAFLD and a critical time point in its progression. The purpose of this study was to analyze the diagnostic accuracy of the non-invasive Fibrotic NASH Index (FNI) in individuals with obesity undergoing bariatric surgery.

**Methods** This is a cross-sectional study, enrolling individuals who underwent bariatric surgery with liver biopsy at a tertiary university hospital. FNI was calculated, and a cutoff value was determined. Its diagnostic accuracy was then calculated through comparison with the gold standard test for this analysis (histopathological examination).

**Results** Of 128 participants, 83.6% were female, and the average age was  $39.8 \pm 8.7$  years. The mean BMI was  $38.7 \pm 5.7$  kg/m<sup>2</sup>. NAFLD was histologically confirmed in 76.6%, of which 81.6% had NASH. Histologically confirmed fibrotic NASH was observed in 22.7% of the general study population, 29.6% of individuals with NAFLD, and 36.3% of those with NASH. The mean FNI was  $0.18 \pm 0.19$ . An optimal cutoff point of 0.21 was determined, with an overall accuracy of 90.1%, an 82.8% sensitivity, a 90.8% specificity, a 72.6% positive predictive value, and a 94.7% negative predictive value.

**Conclusions** FNI provided adequate accuracy in detecting and ruling out fibrotic NASH. Considering the importance of fibrotic NASH within the natural history of NAFLD progression and the fact that this marker uses simple variables, it may be of great importance in high-risk populations, and its external validation and use should be encouraged.

**Keywords** Non-alcoholic fatty liver disease · Fatty liver · Obesity · Bariatric surgery · Liver function tests

## Introduction

Non-alcoholic fatty liver disease (NAFLD) is the metabolically related presence of hepatic steatosis in the absence of another secondary cause for the accumulation of liver fat, such as alcohol use [1–3]. It is divided into simple steatosis and non-alcoholic steatohepatitis (NASH). In simple steatosis, there is no significant inflammation, while NASH is characterized by necroinflammatory abnormalities and

is often indistinguishable from alcoholic liver disease [4]. NAFLD is the most common liver disease worldwide, and it is expected that by 2030, it will be the main indication for liver transplantation [4–6].

The association of NASH with significant liver fibrosis is considered the main prognostic factor in NAFLD, with a strong correlation with liver-related mortality [7–11]. Fibrosis represents a milestone in the progression of the disease, meaning that the inflammatory process caused cellular damage associated with an abnormal regenerative process, albeit not necessarily advanced. Identifying and effectively monitoring this stage of progression is essential to stratifying the risk of long-term liver complications [12]. Thus, the concomitant presence of active inflammation and fibrosis represents a relevant time point in the natural history of NAFLD, in which possible interventions are likely to mitigate the

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inflammatory process and even resolve or, at the very least, interrupt fibrosis worsening.

Among various methods for diagnosing NAFLD, the most accurate is liver biopsy, which is considered the gold standard, providing nuanced histological differentiations. However, as it can be an expensive, invasive, and risky procedure, biopsy ends up being inaccessible for large-scale application in such a high-risk population, especially when percutaneously performed in isolation outside a surgical context. Conversely, intraoperative biopsies are safer and more easily obtained during abdominal surgical procedures [13]. Imaging methods can reliably diagnose NAFLD but are not capable of detecting steatohepatitis or appropriately and nuancedly defining the severity of the disease. Transient elastography can detect liver fibrosis, including in its early stages; however, it is not widely available for most public healthcare facilities in our country [14–17].

Based on these aforementioned facts, non-invasive markers have been developed to predict the severity of NAFLD. These surrogate markers aim to estimate the risk of specific histopathological aspects based on highly available and easily accessible variables [18]. The Fibrotic Non-Alcoholic Steatohepatitis Index (FNI), proposed by Tavaglione et al. [20], seems to be a simple and promising way to detect NASH alongside significant fibrosis. This score varies from 0 to 1 and assesses the probability of NASH complicated by significant fibrosis in individuals with a high probability of NAFLD. The laboratory tests used to calculate it are routinely available (hemoglobin HbA1c, aspartate aminotransferase [AST], and high-density lipoprotein cholesterol [HDL-c]). Considering the critical time point within the natural history of NAFLD represented by fibrotic NASH as a milestone of disease progression, the possibility of its non-invasive detection represents a simple, low-cost, and highly accessible method for detecting this and prompting interventions with the necessary readiness before irreversible changes are established.

This study aimed to analyze the diagnostic accuracy of FNI to detect fibrotic NASH in individuals with obesity undergoing bariatric surgery.

## Methods

### Study Design

A descriptive cross-sectional study was carried out, enrolling individuals who underwent bariatric surgery at a tertiary university hospital. The non-invasive marker FNI was calculated, and its diagnostic accuracy was calculated through comparison with the gold standard test for this analysis (histopathological evaluation of liver biopsy specimens). This study was approved by the local institutional

review board under the opinion 6.327.498 (CAAE: 72859023.5.0000.5404)/FCM-UNICAMP.

### Study Population

This study included individuals aged over 18 years old, undergoing bariatric surgery at this institution between January 2017 and March 2019 who agreed to participate in this protocol by signing an informed consent form. Individuals considered vulnerable (underaged, people with disabilities or mental illness, or those under state tutelage) were excluded, as well as those with other liver diseases, previous or ongoing biliary obstruction, previous or active viral hepatitis, and users of alcohol and/or hepatotoxic medications.

### Liver Biopsy

Wedge liver biopsies are routinely collected during bariatric operations at this hospital. They are performed by extracting a 2-cm fragment of the left hepatic lobe at the end of the procedure.

### Study Variables

Demographic (age and gender), clinical (presence of comorbidities), anthropometric (body mass index – BMI), NAFLD-related histopathological characteristics, and the non-invasive FNI score were analyzed.

### Histopathological Variables

Data on histopathological variables were analyzed by the same pathology team. NAFLD was histopathologically stratified according to the Brunt Classification: steatosis (macro- and/or microvesicular; absent, mild, moderate, and severe); lobular inflammation (0 [absent], 1 + [mild], 2 + [moderate], 3 + [severe]); portal inflammation (0 [absent], 1 + [mild], 2 + [moderate], 3 + [severe]); hepatocellular ballooning (0 [absent]; grades 1 or 2); and fibrosis (0 – absent; 1a – mild perisinusoidal; 1b – moderate perisinusoidal; 1c – isolated periportal; 2 – periportal and perisinusoidal; 3 – presence of bridging fibrosis; 4 – cirrhosis) [7].

The diagnosis of NASH was made through the Matteoni criteria, defined by the concomitant presence of macrovesicular steatosis alongside lobular inflammation and hepatocellular ballooning [18]. The NAFLD activity score (NAS) was also assessed, defined by the sum of intensities of macrovesicular steatosis, lobular inflammation, and hepatocellular ballooning, according to the Pathology Committee of the NASH Clinical Research Network [19].

## Fibrotic NASH Index (FNI)

Tavaglione et al. [20] described this score for screening for fibrotic NASH in primary healthcare. It aimed to identify individuals at high risk for NASH with concomitant fibrosis, including those with overweight/obesity, type 2 diabetes, and metabolic syndrome. Fibrotic NASH was defined as the concomitant presence of histologically proven NASH,  $NAS \geq 4$ , and fibrosis  $\geq F2$ . Using three predictors considered independent of the disease (AST, HDL-c, and hemoglobin HbA1c), the authors derived a model whose calculation is freely available at the website: <<https://fniscore.github.io/>>.

The prediction model for fibrotic NASH was generated and validated internally in the study group “Molecular Architecture of Fatty Liver Disease in Individuals with Obesity Undergoing Bariatric Surgery” (MAFALDA). It was then validated in three more independent cohorts, with appropriate performance and areas under the curve varying from 0.80 to 0.95.

FNI ranges from 0 to 1, being a probability predictor. In the validation cohort, an  $FNI > 0.1$  meant a risk of severe liver disease four times higher than in the general population. The authors stated that the score is mainly intended to rule out fibrotic NASH in primary care, separating individuals with simple forms from those who should be referred for secondary evaluation. They also stated that FNI could be used as a longitudinal biomarker to non-invasively assess the effectiveness of interventional strategies for NASH over time.

## Statistical Analysis

Normality was calculated using the Shapiro-Wilk test. To define the accuracy of the FNI score in this study population, receiver operating characteristic (ROC) curve analysis was performed, the optimal cutoff value was determined through Youden’s method, and diagnostic accuracy variables (sensitivity, specificity, positive and negative predictive values, positive and negative likelihood ratios, and overall accuracy) were then calculated. To graphically plot the ROC curves, the Epitools website (<http://epitools.ausvet.com.au>) was used [21]. A significance level of 5% ( $p < 0.05$ ) was adopted. The computer program Software SAS Release 8.2 (SAS Institute Inc., Cary, NC) was used to perform the analyses.

## Results

Out of 128 individuals included, 83.6% were female, and the average age was  $39.8 \pm 8.7$  years. The main obesity-associated medical conditions observed were hypertension (57%) and type 2 diabetes (27.3%). The mean BMI

was  $38.7 \pm 5.7$  kg/m<sup>2</sup>. In Table 1, there is a complete description of the general characteristics of the study population.

**Table 1** General characteristics of the study population

<i>N</i>	128
Age (years)	$38.4 \pm 9.3$
Gender	M: 21 (12.3%) F: 107 (87.7%)
Obesity-associated medical conditions:	
Hypertension – <i>N</i> (%)	73 (57%)
Type 2 diabetes – <i>N</i> (%)	35 (27.3%)
BMI (kg/m <sup>2</sup> )	$38.7 \pm 5.7$
Liver biopsy findings	
Macrovesicular steatosis – <i>N</i> (%)	98 (76.6%)
Microvesicular steatosis – <i>N</i> (%)	25 (19.5%)
Hepatocellular ballooning – <i>N</i> (%)	75 (58.6%)
Lobular inflammation – <i>N</i> (%)	79 (61.7%)
Portal inflammation – <i>N</i> (%)	55 (58.6%)
Fibrosis – <i>N</i> (%)	82 (64.1%)
1a – <i>N</i> (%)	25 (30.5%)
1b – <i>N</i> (%)	3 (3.7%)
1c – <i>N</i> (%)	1 (1.2%)
2 – <i>N</i> (%)	46 (56.1%)
3 – <i>N</i> (%)	7 (8.5%)
4 – <i>N</i> (%)	0
NAFLD classification (Matteoni’s criteria)	
0	30 (23.4%)
I	15 (11.7%)
II	3 (2.4%)
III	10 (7.8%)
IV	70 (54.7%)
Simple steatosis	52 (30.4%)
NASH	76 (44.4%)
NAS	$2.6 \pm 1.8$
Biochemical variables	
AST (U/L)	$25.9 \pm 16.7$
ALT (U/L)	$30.4 \pm 37.3$
Albumin (g/dL)	$4.3 \pm 0.3$
Glucose (mg/dL)	$91.7 \pm 28.7$
Hemoglobin A1c (%)	$5.6 \pm 1$
Hemoglobin (g/dL)	$13.8 \pm 1.3$
Ferritin (ng/mL)	$214 \pm 205.8$
Serum iron ( $\mu$ g/L)	$69.6 \pm 29.5$
LDL-c (mg/dL)	$104.3 \pm 30$
HDL-c (mg/dL)	$41.8 \pm 9.7$
Triglycerides (mg/dL)	$110.4 \pm 53.4$

*N*, number of individuals; *NAFLD*, non-alcoholic fatty liver disease; *NASH*, non-alcoholic steatohepatitis; *BMI*, body mass index; *AST*, aspartate aminotransferase; *ALT*, alanine aminotransferase; *NAS*, NAFLD activity score; *LDL-c*, low-density lipoprotein; *HDL-c*, high-density lipoprotein

Regarding NAFLD, 76.6% presented with a histologically confirmed diagnosis. The most frequently observed histopathological abnormalities were macrovesicular steatosis (76.6%), lobular inflammation (61.7%), and fibrosis (64.1%). According to Matteoni's criteria, 14.1% of participants had simple steatosis and 62.5% presented with NASH. Among patients diagnosed with NAFLD, 81.6% had NASH. Table 1 presents the complete description of the histological data from the present study. Histologically confirmed fibrotic NASH was observed in 29 participants (22.7% of the general study population, 29.6% of individuals with NAFLD, and 36.3% of those with NASH) (Fig. 1).

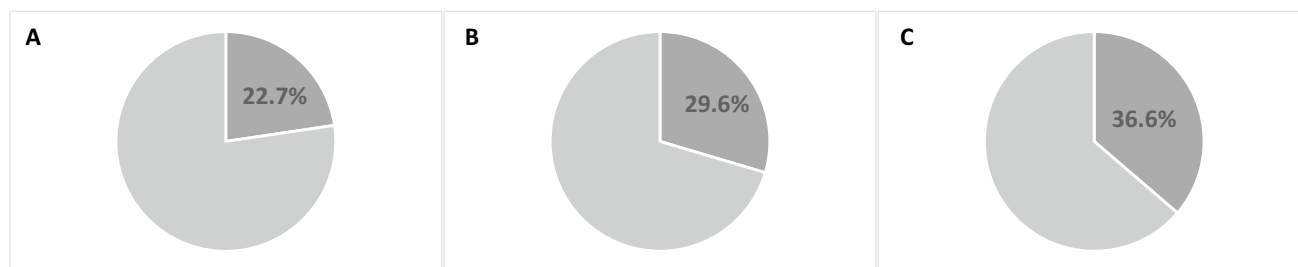
The mean FNI was  $0.18 \pm 0.19$ . After analyzing the ROC curve of the distribution of results, an optimal cutoff point of 0.21 was determined using Youden's method, with an area under the curve (overall accuracy) of 90.1%, an 82.8% sensitivity, and a 90.8% specificity (Figs. 2 and 3). The other diagnostic accuracy variables were then calculated, with a positive predictive value of 72.6%, a negative

predictive value of 94.7%, a positive likelihood ratio of 9, and a negative likelihood ratio of 0.2.

## Discussion

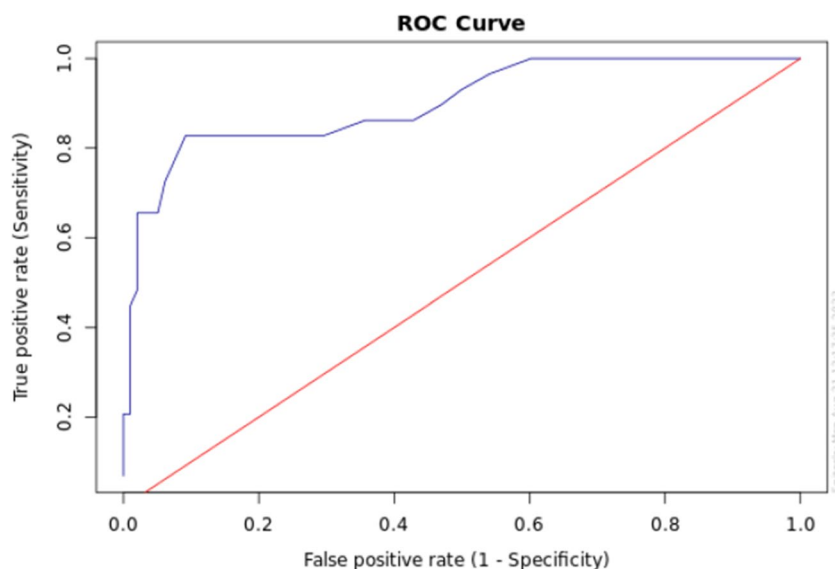
The presence of fibrotic NASH represents a time point of great relevance in the progression of NAFLD. It denotes the coexistence of active hepatic inflammation alongside already established fibrosis, although not necessarily in advanced stages. This phase represents a critical moment in the trajectory of NAFLD, where therapeutic intervention may be essential to promote its regression or, at least, interrupt its progression [13].

A substantial proportion of individuals with confirmed NAFLD among a bariatric population was observed in the present study, reiterating the importance of this disease in obesity. Furthermore, more than 60% of participants and 80% of those with NAFLD had some degree of liver fibrosis, demonstrating the severity of the disease in this

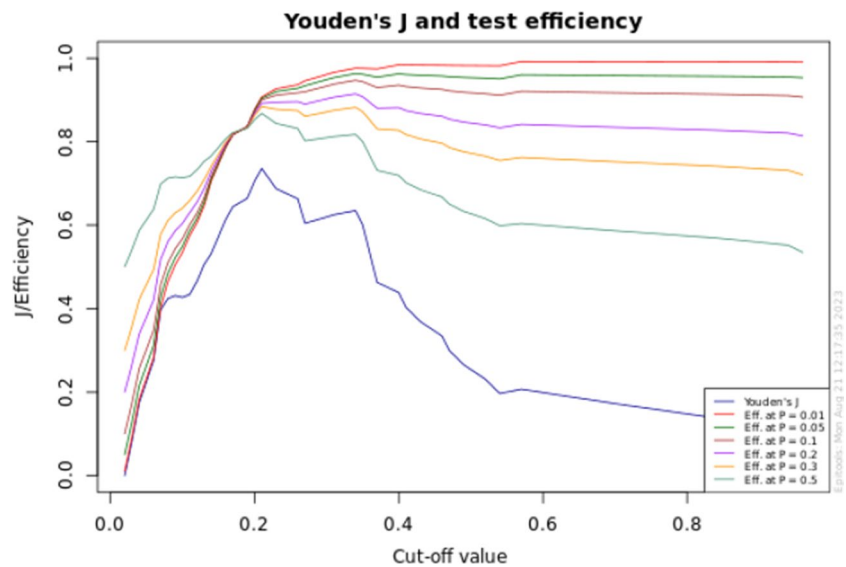


**Fig. 1** Proportion of individuals with fibrotic non-alcoholic steatohepatitis (NASH) in different subsets: (A) entire study population, (B) individuals with non-alcoholic fatty liver disease (NAFLD), and (C) individuals with NASH

**Fig. 2** Graphic representation of the receiver operation characteristics' (ROC) curve analyzing the accuracy of fibrotic non-alcoholic steatohepatitis index (FNI) in the study population



**Fig. 3** Graphic representation of the receiver operation characteristics' (ROC) curve determining the optimal cutoff point of fibrotic non-alcoholic steatohepatitis index (FNI) in the study population through Youden's method



population and corroborating previous evidence that also observed a high rate of fibrosis in patients with obesity and NAFLD [18, 22–24].

The main finding of the current study was the confirmation of fibrotic NASH in more than 20% of the study population. This indicates an advanced and potentially severe phase of NAFLD in this population. Fibrotic NASH is of particular concern because of its association with a significantly increased risk of progression to liver cirrhosis, hepatocellular carcinoma, and their complications [12].

FNI was considered an adequate tool to assess the presence of fibrotic NASH in the individuals included in this study, with appropriate efficacy in both detecting and ruling out fibrotic NASH. After determining an optimal cutoff point of 0.21, a sensitivity of 82.8%, a specificity of 90.8%, a positive predictive value of 72.6%, and a negative predictive value of 94.7% were provided. FNI's high sensitivity meant that it could correctly identify more than 80% of true cases of fibrotic NASH, which is particularly important in this clinical context. Additionally, the specificity above 90% indicated that FNI pointed out a significant capacity to correctly identify individuals without fibrotic NASH; that is, it could reliably rule out this abnormality.

The determination of a unique cutoff value, in this case, 0.21, was important to facilitate and standardize the interpretation of FNI results. This simplifies the clinical application of the test, making it simpler to use in daily practice. Furthermore, a specific cutoff value helps ensure consistency and reproducibility of results, making FNI a reliable and simple tool. It is important to note that the excellent performance of FNI in this study places it in an advantageous position compared to other NAFLD markers or diagnostic methods. FNI has demonstrated its ability to detect fibrosis at a relatively early stage, starting at grade 2 ( $\geq$  F2) [20].

This is a critical time point, as it allows therapeutic intervention at a time when the progression of NAFLD is still a more feasible objective. In comparison, other markers of liver fibrosis, such as AST-to-platelet ratio (APRI), NAFLD Fibrosis Score (NFS), and BMI, AST/ALT Ratio, and Diabetes (BARD) can only detect fibrosis in more advanced stages, greater or equal to stage 3 ( $\geq$  F3), which may result in late diagnoses [22–27].

Hence, it was demonstrated that FNI may play a role in screening patients at high risk for NAFLD, allowing early identification of fibrotic NASH, using simple, highly accessible, and low-cost laboratory tests, even enabling its use on a large scale and in primary care contexts. Identification of these patients is crucial to warranting early interventions, such as bariatric surgery in patients with refractory obesity. Bariatric surgery not only promotes weight loss but also improves metabolic comorbidities and reduces the risk of serious liver complications, significantly contributing to the effective management of NAFLD in high-risk patients [28–31]. Nonetheless, it by no means can replace liver biopsy, since the latter can provide a more thoroughly nuanced and reliable analysis of all histopathological aspects of NAFLD and its current stage within its natural history. There are also other validated non-invasive methods to assess liver fibrosis, such as the Fibrotest and transient elastography. Fibrotest estimates the degree of liver fibrosis and necroinflammatory abnormalities through a formula that encompasses quantitative results of five serum biochemical markers ( $\alpha$ 2-macroglobulin, haptoglobin, apolipoprotein A1, bilirubin, and gamma glutamyl transpeptidase). It can provide results that reliably correlate with histopathological analyses, with a higher than 90% accuracy in its validation cohorts; however, it requires the determination of biochemical examinations that are not widely available, mainly



$\alpha$ 2-macroglobulin and apolipoprotein A1 [32]. A systematic review conducted by Vali et al. also pointed out that Fibrotest is more accurate to detect either advanced fibrosis or cirrhosis in individuals with NAFLD [33]. Transient elastography is also a reliable, non-invasive tool to detect fibrosis within this context. A meta-analysis carried out by Selvaraj et al. demonstrated that magnetic resonance elastography provides an above 90% capacity to detect significant fibrosis ( $\geq$  F2), a somewhat early stage when interventions can be highly desirable [34]. Nevertheless, the main current limitation to a broader use of elastography is its low availability, especially in public healthcare facilities in our country.

The current study has some limitations that need to be considered. Its retrospective design is susceptible to lower-quality data and selection bias. Cross-sectional studies allow determining associations but do not permit establishing cause-and-effect relationships over time. The current study was carried out in a population of patients undergoing bariatric surgery, who make up a more homogeneous sample than the general population in terms of demographic, clinical, and anthropometric characteristics, which limits the generalization of the results to other populations. On the other hand, our findings confirmed the reliability of an easily applicable and highly accessible score and encouraged further research on the use of this marker in populations known to be at high risk for NAFLD.

## Conclusions

In a bariatric population, FNI provided adequate accuracy in detecting and ruling out the presence of fibrotic NASH, which is conceptually an important histopathological landmark within the NAFLD spectrum because it selects individuals who have at least established fibrosis ( $\geq$  F2), albeit not yet necessarily severe, alongside an active inflammatory activity, that is, with a risk of short-to-mid-term progression to severe forms. Considering the importance of fibrotic NASH within the natural history of NAFLD progression and the fact that this marker uses simple variables, it may be of great importance in high-risk populations, and its external validation and use should be encouraged.

## Declarations

**Ethical Approval** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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

**Conflict of Interest** Luísa Souza Echeverria: no conflict of interest.

Daniel Leandro Saran Mounzer: no conflict of interest.  
 Martinho Antonio Gestic: no conflict of interest.  
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