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## Reliability of Liver Stiffness Measurement in Non-alcoholic Fatty Liver Disease: the Effect of Body Mass Index

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**Reliability of Liver Stiffness Measurement in Non-alcoholic  
Fatty Liver Disease: the Effect of Body Mass Index**

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40 **ABBREVIATIONS:** NAFLD : non-alcoholic fatty liver disease; BMI: body mass index ; TE:  
41  
42 transient elastography; LSM: liver stiffness measurement.  
43  
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46  
47  
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## ABSTRACT

**Background:** Liver stiffness measurement (LSM) by transient elastography (TE) is used to stage fibrosis in patients with liver disease, but its diagnostic reliability and the factors affecting its performance in patients with non-alcoholic fatty liver disease (NAFLD) are incompletely understood.

**Methods:** Consecutive NAFLD patients (n=169), assessed by liver biopsy (Kleiner score), anthropometric, biochemical and metabolic features, underwent LSM by TE with standard M probe.

**Results:** LSM was not reliable in 23 patients (13.6%), due to obesity. Among patients with a reliable TE, a LSM value >7.25 KPa was the best cut-off for predicting significant fibrosis at biopsy (AUC 0.794), yet this cut-off still failed to rule out F2-F4 fibrosis in 30.8% (false-negative rate) or rule in F3-F4 in 29.4% (false-positive rate). Similarly a LSM value >8.75 KPa was the best cut-off for severe fibrosis (F3-F4) (AUC 0.870), with a rate of false-negatives of 24.2% and of false-positives of 2.1%. Body mass index was the major determinant of these diagnostic errors in predicting significant and severe fibrosis both by overestimating or underestimating the stage of fibrosis.

**Conclusions:** In NAFLD patients, even when LSM is feasible, high BMI values affect negatively the diagnostic reliability. Improved performance of TE could be obtained using specifically designed probes.

## INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is one of the most common chronic liver diseases worldwide, with a prevalence of about 20%-30% [1]. A relevant proportion of NAFLD patients, particularly those with non-alcoholic steatohepatitis (NASH), may progress to cirrhosis and its complications [2]. Anthropometric factors [3] and liver necroinflammation [4] are associated with the progression of liver disease over time, even if the severity of fibrosis is the strongest predictor of liver related morbidity and mortality [5]. Along this line, the evaluation of liver fibrosis is crucial for the prognostic evaluation of NAFLD patients, particularly in patients without advanced disease, but at risk of developing cirrhosis, like those with significant or severe fibrosis.

Biopsy, even if invasive, painful, and with potentially life-threatening complications, remains the gold standard for the evaluation of liver fibrosis [6,7]. In the past few years different non-invasive biochemical and instrumental tools have been used, with contrasting results [8]. In this setting various number of studies have proposed liver stiffness measurement (LSM) by transient elastography (TE) as a quick, accurate and non-invasive test to assess the stage of fibrosis and portal hypertension in patients with chronic liver disease of different aetiologies [9]. Most of these study were performed in patients with chronic hepatitis C, while limited data are available in subjects with NAFLD. Recent studies on children [10] and adults [11-15] with NAFLD have shown a good performance of LSM in staging fibrosis. Two of these studies also identified severe steatosis [13,14] as a factor reducing the reliability of TE, while an insufficient length of the liver biopsy may underestimate the stage of fibrosis more correctly classified by TE [12]. A major limiting factor to the diagnostic performance of TE in NAFLD is obesity, which may hinder the execution of TE especially when using the standard M probe.

We aimed to assess in a cohort of consecutive patients with biopsy-proven NAFLD:

- 1) the reliability of LSM, 2) its performance in diagnosing significant and severe fibrosis, and 3) which factors may affect its diagnostic performance.

## **MATERIALS & METHODS**

### **Patients**

The study assessed consecutive patients with biopsy-proven NAFLD, recruited at the GI & Liver Unit of the University Hospital in Palermo, and fulfilling all inclusion and exclusion criteria detailed below. The diagnosis of NAFLD was based on chronically elevated ALT for at least 6 months, alcohol consumption <20 g/day in the last year, steatosis (≥5% of hepatocytes) at histology with/without necroinflammation and/or fibrosis. Exclusion criteria were as follows: (1) advanced cirrhosis (Child-Turcotte-Pugh B and C); (2) hepatocellular carcinoma; (3) other causes of liver disease or mixed etiologies (alcohol abuse, hepatitis C, hepatitis B, autoimmune liver disease, Wilson's disease, haemochromatosis, or  $\alpha$ 1-antitrypsin deficiency); (4) human immunodeficiency virus infection; (5) previous treatment with immunosuppressive drugs, and/or regular use of steatosis-inducing drugs, evaluated by a questionnaire (for example, corticosteroid, valproic acid, tamoxifen, amiodarone); or (6) active intravenous drug addiction or use of cannabis.

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

### **Clinical and laboratory assessment**

Clinical and anthropometric data were collected at the time of liver biopsy. BMI was calculated on the basis of weight in kilograms and height (in meters), and subjects were

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2  
3 classified as normal weight (BMI, 18.5-24.9 kg/m<sup>2</sup>), overweight (BMI, 25-29.9), obese (BMI  
4 ≥30). The diagnosis of arterial hypertension was based on the following criteria: systolic  
5 blood pressure ≥135 mm Hg and/or diastolic blood pressure ≥85 mm Hg (measured three  
6 times within 30 minutes, in the sitting position and using a brachial sphygmomanometer) or  
7 ongoing antihypertensive pharmacological treatment after a diagnosis of arterial  
8 hypertension. The diagnosis of type 2 diabetes was based on the revised criteria of the  
9 American Diabetes Association, using a value of fasting blood glucose at least 126 mg/dL  
10 on at least two occasions [16]. In patients with a previous diagnosis of type 2 diabetes,  
11 current therapy with insulin or oral hypoglycemic agents was documented. A 12-hr  
12 overnight fasting blood sample was drawn at the time of biopsy to determine serum levels  
13 of ALT,  $\gamma$ -glutamyltransferase ( $\gamma$ -GT), total cholesterol and HDL-cholesterol, triglycerides,  
14 ferritin, plasma glucose concentration, insulin levels and platelet count. IR was determined  
15 by the homeostasis model assessment (HOMA) method [17].

### 34 **Histology**

35  
36 Slides were coded and read by one pathologist (D.C.) unaware of the patient's  
37 identity and history. A minimum length of 15 mm of biopsy specimen or the presence of at  
38 least 10 complete portal tracts was required [18]. The percentage of hepatocytes  
39 containing macrovesicular fat was determined for each 10x field. An average percentage  
40 of steatosis was then determined for the entire specimen. Kleiner's classification [19] was  
41 used to compute the NAFLD activity score (from 0 to 8, on a scale including separate  
42 scores for steatosis, lobular inflammation, and hepatocellular ballooning) and to stage  
43 fibrosis from 0 to 4.

### 55 **Liver Stiffness Measurement**

56  
57 TE was performed with the FibroScan (Echosens, Paris, France) medical device using the  
58 M probe (also named the standard probe) to measure LSM. LSM was performed on the  
59 same day of liver biopsy, before the procedure.  
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3 The only operator was a staff physician (G.B.) who had previously performed at  
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5 least 100 determinations in patients with chronic liver disease. The median value of 10  
6  
7 successful acquisitions, expressed in kilopascal (KPa), was maintained as representative  
8  
9 of LSM. As previously described in the literature [20], and as suggested by the  
10  
11 manufacturing company, we considered 10 successful acquisitions with a success rate of  
12  
13 at least 60%, and with an interquartile range lower than 20%, as representative  
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15 measurements.  
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## 19 20 **Statistics**

21  
22 Continuous variables were summarized as mean  $\pm$  SD, and categorical variables as  
23  
24 frequency and percentage. The Student's t-test was used when appropriate. Multiple  
25  
26 logistic regression models were used to assess the relationship of fibrosis to demographic,  
27  
28 virological, metabolic, instrumental and histological characteristics of patients. In this  
29  
30 model, the dependent variables were significant fibrosis, coded as 0 = F0 to F1 in the  
31  
32 fibrosis score or 1 = F2 to F4 in the fibrosis score; severe fibrosis coded as 0 = F0 to F2 in  
33  
34 the fibrosis score or 1 = F3 to F4 in the fibrosis score. As candidate risk factors we  
35  
36 selected age, gender, BMI, baseline ALT, platelet count levels,  $\gamma$ -GT levels, ferritin, total-  
37  
38 cholesterol, HDL cholesterol, triglycerides, blood glucose, insulin, HOMA score, arterial  
39  
40 hypertension, diabetes, steatosis grade, lobular inflammation, hepatocellular ballooning,  
41  
42 NAS score, and LSM.  
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48 Receiver operating characteristic (ROC) curves were applied to find the best cut-off  
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50 values, and to identify the area under ROC curve (AUC) of the LSM able to discriminate  
51  
52 the different classes of fibrosis. Finally, multiple logistic regression models for LSM, using  
53  
54 the best LSM cut-offs for discriminating significant fibrosis, and severe fibrosis as the  
55  
56 dependent variables were performed.  
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60 Variables found to be associated with the dependent variable at univariate analyses  
(probability threshold,  $p \leq 0.10$ ) were included in all multivariate regression models. To



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2  
3 avoid the effect of co-linearity, diabetes, HOMA-score, blood glucose levels and insulin  
4 levels, as well as steatosis grade, lobular inflammation, hepatocellular ballooning and NAS  
5 score, were not included in the same multivariate model. Regression analyses were  
6 performed using PROC LOGISTIC, PROC REG and subroutine in SAS (SAS Institute,  
7 Inc., Cary, North Carolina, U.S.A.) [21].  
8  
9

## 16 RESULTS

### 18 Patient features and histology

20 From January 2006 to December 2010, we included 169 consecutive patients with NAFLD  
21 underwent transient elastography and liver biopsy. Twenty-three patients (13.6%) failed to  
22 obtain 10 valid LSM acquisitions due to obesity. Hence 146 patients with valid LSM  
23 acquisitions could be included in the performance analysis. Baseline features of these 146  
24 patients are shown in Table 1. The mean BMI was  $29.1 \pm 4.1$  Kg/m<sup>2</sup>. Overall, 21 of 146  
25 patients (14.4%) had normal weight, 71 of 146 (48.6%) were overweight, 54 of 146 (37%)  
26 were obese.  
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29 The mean length of the liver fragments given for histology was 17 mm (range 15–  
30 31), and the mean number of complete portal tracts in the specimens was 12. According  
31 to Kleiner et al.(39) 63% of NAFLD were classified as NASH, 9.6% as non-NASH, and  
32 27.4% were indeterminate. Significant and severe fibrosis were observed in 46% (68/146)  
33 and 22.6% (33/146), respectively.  
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36 Patients who failed LSM acquisitions (8 males, 15 females) had higher BMI  
37 (35.7±6.3 versus 29.1±4.1 kg/m<sup>2</sup>,  $P < 0.001$ ), and showed a not significantly different  
38 prevalence of significant (14/23, 60.8%;  $p=0.20$ ) and severe (8/23, 34.7%;  $p=0.20$ ) fibrosis,  
39 respectively.  
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### 59 Risk factors for fibrosis

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3 Univariate and multivariate analyses were performed to identify potential  
4 associations between each feature and fibrosis at two cut-off levels: a) significant fibrosis  
5 (F2-F4); and b) severe fibrosis (F3-F4). Variables associated to cirrhosis (F4) were not  
6 evaluated due to the low number of cirrhotic patients.  
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12 Older age, female sex, high BMI, high triglycerides, high blood glucose, insulin and  
13 HOMA, diabetes, high LSM, lobular inflammation, hepatocellular ballooning and non-  
14 alcoholic steatohepatitis activity score (NAS) were all linked to severe liver fibrosis. By  
15 multivariate analysis, higher LSM (OR 1.405; 95%CI 1.167-1.692;  $p < 0.001$ ) and high NAS  
16 score (OR 2.168; 95%CI 1.486-3.162;  $p < 0.001$ ) were independently linked to significant  
17 fibrosis (Table 2).  
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26 Older age, female sex, low platelet count, high blood glucose, insulin and HOMA,  
27 high LSM, lobular inflammation, hepatocellular ballooning and NAS score were all linked to  
28 severe fibrosis. Multivariate logistic regression analysis showed that only the following  
29 factors were independently associated with severe fibrosis: higher LSM (OR 1.280; 95%CI  
30 1.094-1.497;  $p = 0.002$ ) and high NAS score (OR 4.060; 95%CI 1.478-11.149;  $p = 0.007$ )  
31 (Supporting document 1).  
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### 41 **Liver Stiffness Measurement**

42 The mean LSM value was 8.9 KPa (range 3.5-47.2). Liver stiffness values  
43 increased significantly according to the stage of fibrosis (figure 1).  
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48 Interestingly, we observed a fair and a moderate agreement between LSM and liver  
49 biopsy, in diagnosing significant (kappa= 0.396; C.I. 0.246-0.545) and severe (kappa=  
50 0.454; C.I. 0.290-0.618) fibrosis, respectively.  
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55 ROC curves identified the best cut-offs of LSM at 7.25 KPa for the diagnosis of  
56 significant fibrosis (AUC 0.794; Sensitivity 69.1%, Specificity 70.5%), and at 8.75 KPa for  
57 severe fibrosis (AUC 0.870; Sensitivity 75.8%, Specificity 77.9%).  
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3 At the LSM cut-off of 7.25 KPa for significant fibrosis, the false-positive rate was  
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5 29.4% (23/78) and the false-negative rate was 30.8% (21/68). Concerning the  
6  
7 performance of LSM > 8.75 KPa for severe fibrosis, the false-negative rate was 24.2%  
8  
9 (8/33) and the false-positive rate was 22.1% (25/113). Figure 2 showed the reliability and  
10  
11 the performance of LSM in diagnosing significant (Figure 2A) and severe (Figure 2B)  
12  
13 fibrosis in overall population.  
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16  
17 Along with these data, factors potentially associated with stiffness cut-offs for  
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19 significant fibrosis (7.25 KPa), and severe fibrosis (8.75 KPa) were assessed.  
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22 LSM > 7.25 KPa was associated with stage of fibrosis (OR 2.175; 95%CI 1.359 -  
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24 3.481;  $p = 0.001$ ), and higher BMI (OR 1.257; 95%CI 1.107 - 1.426;  $p < 0.001$ ) by  
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26 multivariate analysis (Table 3). When replacing in the model BMI with WC, the latter was  
27  
28 independently associated (OR 1.066; 95%CI 1.019 - 1.116;  $p = 0.006$ ) with LSM > 7.25  
29  
30 KPa (Supporting document 2). The ROC curve identified a BMI of 28.7 as the best cut-off  
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32 value for LSM > 7.25KPa. The false-positive rate was higher in patients with BMI>28.7  
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34 (18/31; 58%), compared to those with BMI  $\leq$ 28.7 (5/47; 10.6%) (Figure 3A). By contrast  
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36 the likelihood of having a false-negative result was higher in patients with BMI  $\leq$ 28.7  
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38 (12/23; 52.1%), compared to those with BMI>28.7 (9/45; 20%) (Figure 3B).  
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43 Similarly, multivariate analysis showed that LSM > 8.75 KPa was associated with  
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45 stage of fibrosis (OR 2.315; 95%CI 1.459-3.675;  $p < 0.001$ ), and higher BMI (OR 1.190;  
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47 95%CI 1.056-1.340;  $p = 0.004$ ) (supporting document 3). When replacing in the model BMI  
48  
49 with WC, the latter was independently associated (OR 1.068; 95%CI 1.015 - 1.123;  $p$   
50  
51  $= 0.01$ ) with LSM > 8.75 KPa (Supporting document 4). ROC curve identified a BMI >29.2  
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53 as the best cut-off value for LSM > 8.75 KPa. The probability of having a false-positive  
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55 result was higher in patients with BMI >29.2 (18/47; 38.3%), respect to those with  
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57 BMI  $\leq$ 29.2 (7/66; 10.6%) (Figure 4A). By converse, a comparable rate of false negatives  
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3 was observed in patients with BMI>29.2 (5/20; 25%), and in those with BMI≤29.2 (3/13;  
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5 23%) (Figure 4B).  
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8 Finally, it is noteworthy that when we also included patients who failed LSM, the  
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10 performance of LSM for the diagnosis of both significant (AUC 0.613; S.E. 0.045; 95% C.I.  
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12 0.525-0.701) and severe (AUC 0.647; S.E. 0.059; 95% C.I. 0.532-0.762) fibrosis was not  
13  
14 acceptable.  
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## 18 **DISCUSSION**

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20 In this study of 169 NAFLD patients, mostly overweight or obese and with a low  
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22 prevalence of cirrhosis, we found that LSM, measured by the standard M probe, is  
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24 measurable in about 85% of patients, and we showed that it does not perform well for a  
25  
26 relevant number of patients in diagnosing significant and severe fibrosis. Importantly, we  
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28 found that high BMI values, independently from other variables, reduced the performance  
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30 of LSM in evaluating the stage of fibrosis.  
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35 Due to the increasing prevalence of NAFLD and to the increasing need for non-  
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37 invasive tools to predict the stage of fibrosis in these patients, two main groups of  
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39 non - invasive methodologies for the evaluation of hepatic fibrosis have been developed  
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41 [22,23]. The first group, defined "serum markers", is aimed at predicting fibrosis stage and,  
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43 possibly, other prognostic information, using parameters measurable in serum. Among  
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45 them we can distinguish indirect and direct markers [22,23]. Indirect markers are based on  
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47 single or algorithmic elaboration of commonly observed alterations in liver function that  
48  
49 do not necessarily reflect extracellular matrix metabolism. Direct markers reflect actual  
50  
51 extracellular matrix turnover within the liver: total amount of matrix, matrix deposition,  
52  
53 matrix removal, caspase activity. The second group includes methodologies derived from  
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55 elaboration of parameters obtainable with the current liver imaging techniques (ultrasound,  
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57 computed tomography (CT) scan, magnetic resonance) or to the innovative use of  
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3 principles of physics [22,23]. In this line some groups have recently proposed to use LSM  
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5 by TE in NAFLD patients [10-15].  
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8 In our study, TE was unreliable in about 15% of NAFLD patients due to obesity, this  
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10 result being in line with the range of 5%-10% reported in literature on adult NAFLD  
11  
12 patients [11-15] when using the standard M probe.  
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15 When there were no technical limitations to obtaining reliable TE values, overall  
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17 LSM was acceptable in diagnosing significant fibrosis and good for severe fibrosis, two  
18  
19 conditions at high risk of cirrhosis development. In particular we identified LSM values of  
20  
21 7.25 KPa, and 8.75 KPa as the best cut-offs for discriminating significant fibrosis (F2-F4)  
22  
23 and severe fibrosis (F3-F4) respectively. These stiffness cut-off values were quite  
24  
25 comparable to those identified in other studies [10-15]. Some variability may be ascribed to  
26  
27 the different prevalence of significant fibrosis and of factors affecting liver stiffness  
28  
29 independently from fibrosis in the various study cohorts.  
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34 A relevant issue emerging from our data is that in NAFLD patients BMI may  
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36 interfere with the diagnostic performance of LSM, causing under or overestimation of  
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38 fibrosis as assessed by histology. It is conceivable that in overweight and obese patients,  
39  
40 due to a higher skin-to-liver distance of the hepatic capsula, the presence of non-hepatic  
41  
42 tissue in the volume explored by M probe might bias the results. A relation between high  
43  
44 BMI and higher values of liver stiffness was observed in apparently healthy subjects [24].  
45  
46 In addition Gaia and colleagues [14] recently reported this relationship in a cohort of 72  
47  
48 NAFLD patient, although they failed to demonstrate that, in clinical practice, BMI can under  
49  
50 or overestimate the prediction of liver fibrosis by LSM. The low number of patients enrolled  
51  
52 and differences in the mean values of BMI could explain the difference with our study. In  
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54 fact, in our patients, using a cut-off of 7.25 KPa for significant fibrosis the false-negative  
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56 rate (the proportion of patients with stiffness of  $\leq 7.25$ , but with significant fibrosis) was  
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58 30.8%, and the false-positive rate (the proportion of patients with stiffness  $> 7.25$ , but  
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3 without significant fibrosis) was 29.4%. Similarly, at the cut-off of 8.75 for severe fibrosis,  
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5 we observed a rate of false negative and false positive results of 24.2% and 22.1%,  
6  
7 respectively. However, when considering BMI as independent confounder for LS, we  
8  
9 observed that the false-positive rate for significant fibrosis decreased from 58% in patients  
10  
11 with BMI>28.7 to 10.6% in those with BMI≤28.2, while the false-negative rate was lower  
12  
13 (20%) in the group with BMI>28.7 and higher (51.2%) in patients with BMI≤28.7. Similarly,  
14  
15 at the cut-off of 8.75 KPa for severe fibrosis, the false-positive rate increased from 10.6%  
16  
17 among subjects in with BMI≤29.2 to 38.3% in their counterpart with BMI>29.2; conversely  
18  
19 false-negative results were not different according to BMI distribution. The use of lower  
20  
21 BMI thresholds could reduce the rate of false positive results, however, considering high  
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23 BMI values observed in NAFLD populations, this strategy does not appears useful in  
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25 clinical practice.  
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32 Recent studies on patients with chronic hepatitis C or B [25,26] have also  
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34 underscored that liver inflammation, expressed by necroinflammatory hepatic activity or  
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36 ALT levels, contributes significantly to hepatic stiffness, interfering with the performance of  
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38 TE in staging fibrosis. However in our study, like in the others on NAFLD patients [10-15],  
39  
40 we did not observed this relation, probably due to the lower inflammatory grade observed  
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42 in NAFLD than in viral hepatitis.  
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45  
46 Finally we failed to show a relation between LSM and steatosis. In this setting  
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48 contrasting results exist: some studies did not found any association [11,12,15], while  
49  
50 other found an inverse [14] or a direct [13] relation. The higher mean BMI value and the  
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52 higher prevalence of steatosis >33% in our population, compared to those observed in the  
53  
54 above cited studies could explain these discrepancies.  
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57  
58 The study has limitations. First, the analysis was carried out using a conventional M probe  
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60 for LSM evaluation. A recent study [27] reported that the use of the recently introduced XL  
probe in obese patients reduces the unreliability of the test, obtaining stiffness values

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3 slightly but significantly lower than those obtained using the M probe. These data could  
4  
5 partially mitigate the criticisms of our work about reliability and performance of LSM in  
6  
7 NAFLD patients, even if these results have been obtained in patients with clinical  
8  
9 diagnosis of NAFLD, not demonstrating the superiority of XL probe in staging fibrosis.  
10  
11 Second, the study was generated in a relatively small number of patients, and it will be  
12  
13 interesting to see if these results also hold true in larger groups of patients with NAFLD.  
14  
15 Third, our study included a cohort of European non-drinker patients with low prevalence of  
16  
17 cirrhosis, who were enrolled in a tertiary referral center for liver disease, limiting the broad  
18  
19 application of the results. In addition another limit of the present study could reside in the  
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21 accuracy of liver biopsy examination for assessing fibrosis, related to sampling errors,  
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23 technical processing of the specimens, intra- and inter-observer variability and both to the  
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25 length of biopsy specimens and the number of portal spaces. A further methodological  
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27 issue could reside in the accuracy of liver biopsy examination for assessing fibrosis,  
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29 related to sampling errors, technical processing of the specimens, intra- and inter-observer  
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31 variability and both to the length of biopsy specimens and the number of portal spaces.  
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33 However, we are confident that the minimum length of 15 mm, and the presence of at least  
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35 10 complete portal tracts, minimize this bias. Finally, we cannot exclude the possibility that  
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37 hidden abuse of alcohol may be responsible for the presence of stiffness variations in a  
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39 few subjects.  
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48 In conclusion, we believe that, even if we have provided further data for a more  
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50 correct interpretation of LSM in NAFLD patients, the evidence is sufficient to consider that  
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52 in this setting LSM by standard M probe does not perform well for most patients in  
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54 diagnosing significant and severe fibrosis. In fact, in NAFLD patients, high BMI values, not  
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56 only reduce the feasibility of TE but also affect its performance by over or underestimate  
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58 the stage of fibrosis. In addition, considering the higher prevalence of obesity in NAFLD  
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60 general population, compared to when observed in our cohort, it should be hypothesized



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3 an even lower performance of LSM in this clinical setting. According to the above, we  
4 suggest that LSM alone is an useful, though not sufficient, tool for staging liver fibrosis in a  
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6 low number of NAFLD patients, and that the use of specifically developed probes for larger  
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8 patients will lead to a better reliability and performance of TE.  
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### Contributors.

S. Petta designed the study, contributed to data acquisition, was responsible for writing the manuscript, and participated in statistical analysis. C. Cammà participated in the writing of the manuscript and was responsible for statistical analysis. V. Di Marco , and A. Craxì (Director of the GI & Liver Unit) were responsible for writing the manuscript and have seen and approved the final version. G. Butera and D. Cabibi participated in patient management and data collection. All authors have seen and approved the final version of the manuscript.

### Figure Legends

**Figure 1.** Distribution of liver stiffness values according to the different stages of fibrosis

**Figure 2.** Reliability and prediction of significant fibrosis (A) and severe fibrosis (B) in patients with NAFLD, by using transient elastography.

**Figure 3.** Distribution of false-positive cases in patients without significant fibrosis (A), and of false-negative cases in patients with significant fibrosis (B), according to BMI cut-off of 28.7.

**Figure 4.** Distribution of false-positive cases in patients without severe fibrosis (A), and of false-negative cases in patients with severe fibrosis (B), according to BMI cut-off of 29.2.

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17 **Table 1.** Demographic, laboratory, metabolic and histological features of 146 consecutive patients with non-alcoholic  
18 fatty liver disease.  
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For Peer Review

Data are given as mean  $\pm$  SD or as number of cases (%). yrs indicates years; HDL, high-density lipoprotein; and

| Variable                                 | Nonalcoholic Fatty Liver Disease<br>(n=146) |
|--|---|
| Mean age – yrs                           | 44.1 $\pm$ 13.2                             |
| Gender<br>male/ female                   | 104 (71.2)/42 (28.8)                        |
| Mean body mass index – kg/m <sup>2</sup> | 29.1 $\pm$ 4.1                              |
| Body mass Index – kg/m <sup>2</sup>      |   |
| <25                                      | 21 (14.4)                                   |
| 25-29.9                                  | 71 (48.6)                                   |
| $\geq$ 30                                | 54 (37.0)                                   |
| Alanine aminotransferase – IU            | 80.9 $\pm$ 57.8                             |
| $\gamma$ glutamyl transferase – IU       | 111.7 $\pm$ 148.2                           |
| Ferritin – ng/mL                         | 273.7 $\pm$ 270.0                           |
| Cholesterol – mg/dL                      | 203.6 $\pm$ 48.5                            |
| HDL cholesterol – mg/dL                  | 48.1 $\pm$ 16.6                             |
| Triglycerides – mg/dL                    | 152.1 $\pm$ 81.2                            |
| Blood glucose – mg/dL                    | 97.6 $\pm$ 31.5                             |
| Insulin – $\mu$ U/mL                     | 17.0 $\pm$ 10.9                             |
| HOMA-score                               | 4.30 $\pm$ 3.79                             |
| Diabetes<br>absent/present               | 126(86.3)/20 (13.7)                         |
| Hypertension<br>absent/present           | 120(82.2)/26 (17.8)                         |
| Liver Stiffness – Kpa                    | 8.9 $\pm$ 7.0                               |
| <b>Histology</b>                         |   |
| <b>NAFLD activity score (NAS)</b>        |   |
| 0-2                                      | 14 (9.6)                                    |
| 3-4                                      | 40 (27.4)                                   |
| 5-8                                      | 90 (63.0)                                   |
| <b>Lobular inflammation</b>              |   |
| 0  | 10 (6.8)                                    |
| 1  | 71 (48.6)                                   |
| 2  | 60 (41.2)                                   |
| 3  | 5 (3.4)                                     |
| <b>Steatosis as continuous variable</b>  | 45.4 $\pm$ 26.6                             |
| <b>Steatosis grade</b>                   |   |
| 1 (5%-33%)                               | 55 (37.7)                                   |
| 2 (>33%-66%)                             | 42 (28.8)                                   |
| 3 (>66%)                                 | 49 (33.5)                                   |
| <b>Hepatocellular ballooning</b>         |   |
| 0  | 13 (8.9)                                    |
| 1  | 53 (36.3)                                   |
| 2  | 80 (54.8)                                   |
| <b>Stage of Fibrosis</b>                 |   |
| 0  | 38 (26.0)                                   |
| 1  | 40 (27.4)                                   |
| 2  | 35 (24.0)                                   |
| 3  | 22 (15.1)                                   |
| 4  | 11 (7.5)                                    |

HOMA, homeostasis model assessment.

**Table 2. Univariate and multivariate analysis of risk factors associated with significant fibrosis (F2-F4) in 146 patients with non-alcoholic fatty liver disease.**

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| Variable                                 | No Significant Fibrosis<br>N=78 | Significant Fibrosis<br>n=68 | Univariate<br>Analysis<br><i>p</i> value | Multivariate Analysis<br>OR (95% CI) | <i>p</i> value |
|--|---------------------------------|------------------------------|--|--------------------------------------|----------------|
| Mean age – yrs                           | 41.2 ± 11.6                     | 47.5 ± 14.2                  | 0.004                                    | 1.007 (0.974 – 1.042)                | 0.67           |
| Gender<br>male/ female                   | 65/13                           | 39/29                        | <0.001                                   | 2.240 (0.843 – 5.956)                | 0.10           |
| Mean body mass index – kg/m <sup>2</sup> | 28.5 ± 4.3                      | 29.9 ± 3.6                   | 0.03                                     | 0.922 (0.816 - 1.043)                | 0.19           |
| Waist circumference- cm                  | 96.2 ± 12.1                     | 100.1 ± 11.7                 | 0.08                                     | -                                    |                |
| Alanine aminotransferase – IU            | 83.4 ± 63.2                     | 78.1 ± 51.2                  | 0.58                                     | -                                    |                |
| γ glutamyl transferase – IU              | 98.7 ± 117.7                    | 126.7 ± 176.6                | 0.25                                     | -                                    |                |
| Platelet count- 10 <sup>3</sup> mmc      | 222.0 ± 50.8                    | 212.3 ± 73.7                 | 0.35                                     | -                                    |                |
| Ferritin – ng/mL                         | 265.7 ± 241.9                   | 283.2 ± 301.2                | 0.70                                     | -                                    |                |
| Cholesterol – mg/dL                      | 203.3 ± 44.4                    | 203.8 ± 53.0                 | 0.94                                     | -                                    |                |
| HDL cholesterol – mg/dL                  | 50.0 ± 16.9                     | 46.0 ± 16.0                  | 0.14                                     | -                                    |                |
| Triglycerides – mg/dL                    | 140.1 ± 76.3                    | 165.8 ± 84.9                 | 0.05                                     | 1.001 (0.995 - 1.007)                | 0.69           |
| Blood glucose – mg/dL                    | 92.0 ± 22.6                     | 104.1 ± 38.5                 | 0.02                                     | -                                    |                |
| Insulin – μU/mL                          | 13.7 ± 8.0                      | 20.7 ± 12.4                  | <0.001                                   | -                                    |                |
| HOMA-score                               | 3.26 ± 2.87                     | 5.49 ± 4.35                  | <0.001                                   | 0.988 (0.845 – 1.156)                | 0.88           |
| Diabetes                                 | 73/5                            | 53/15                        | 0.006                                    |                                      |                |
| Arterial Hypertension                    | 67/11                           | 53/15                        | 0.21                                     |                                      |                |
| Liver Stiffness - KPa                    | 6.4 ± 2.2                       | 11.8 ± 9.3                   | <0.001                                   | 1.405 (1.167 - 1.692)                | <0.001         |
| <b>Histology</b>                         |                                 |                              |  |                                      |                |
| <b>NAS score</b> 0-2/3-4/5-8             | 14/30/34                        | 0/10/58                      | <0.001                                   | 2.168 (1.486 – 3.162)                | <0.001         |
| <b>Lobular inflammation</b> 0/1/2/3      | 10/49/19/0                      | 0/22/41/5                    | <0.001                                   | -                                    |                |
| <b>Steatosis (continuous variable)</b>   | 40.3 ± 27.9                     | 51.3 ± 23.8                  | 0.01                                     | -                                    |                |
| <b>Steatosis grade</b> 0/1/2/3           | 0/35/22/21                      | 0/20/20/28                   | 0.03                                     | -                                    |                |
| <b>Hepatocellular ballooning</b> 0/1/2   | 12/35/31                        | 1/18/49                      | <0.001                                   | -                                    |                |

Data are given as mean ± SD or as number of cases (%). yrs indicates years; HDL, high-density lipoprotein; HOMA, homeostasis model assessment; and NAS, non-alcoholic steatohepatitis activity score.

**Table 3. Univariate and multivariate analysis of factors associated with liver stiffness >7.25 Kpa for significant fibrosis in 146 patients with non-alcoholic fatty liver disease.**

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| Variable                                    | Liver Stiffness<br>≤ 7.25 Kpa<br>n=76 | Liver Stiffness<br>> 7.25 Kpa<br>n=70 | Univariate<br>Analysis<br>p value | Multivariate Analysis<br>OR (95% CI) | P value |
|---|---------------------------------------|---------------------------------------|-----------------------------------|--------------------------------------|---------|
| Age – yrs                                   | 41.3 ± 10.9                           | 47.2 ± 14.8                           | 0.007                             | 1.002 (0.968 – 1.036)                | 0.92    |
| Gender<br>Male vs. Female                   | 61/15                                 | 43/27                                 | 0.01                              | 1.458 (0.522 – 4.072)                | 0.47    |
| Body Mass Index – Kg/m <sup>2</sup>         | 27.3 ± 3.4                            | 31.1 ± 3.8                            | <0.001                            | 1.257 (1.107 – 1.426)                | <0.001  |
| Waist Circumference- cm                     | 94.0 ± 9.8                            | 102.6 ± 12.8                          | <0.001                            | -                                    |         |
| Alanine aminotransferase – IU               | 85.5 ± 66.1                           | 76.0 ± 47.1                           | 0.32                              | -                                    |         |
| Platelet count- 10 <sup>3</sup> mmc         | 222.5 ± 53.1                          | 211.9 ± 71.2                          | 0.30                              | -                                    |         |
| γ glutamyl transferase – IU                 | 109.0 ± 131.5                         | 114.7 ± 165.3                         | 0.82                              | -                                    |         |
| Ferritin – ng/mL                            | 256.2 ± 231.2                         | 293.7 ± 308.8                         | 0.40                              | -                                    |         |
| Cholesterol – mg/Dl                         | 205.8 ± 47.7                          | 201.1 ± 49.4                          | 0.56                              | -                                    |         |
| HDL Cholesterol – mg/Dl                     | 50.7 ± 19.1                           | 45.3 ± 12.9                           | 0.05                              | 0.999 (0.968 - 1.031)                | 0.95    |
| Triglycerides – mg/Dl                       | 143.2 ± 79.1                          | 161.6 ± 82.3                          | 0.17                              | -                                    |         |
| Blood glucose – mg/dL                       | 86.8 ± 12.0                           | 109.4 ± 40.7                          | <0.001                            | -                                    |         |
| Insulin – μU/mL                             | 13.0 ± 6.7                            | 21.2 ± 12.8                           | <0.001                            | -                                    |         |
| HOMA-score                                  | 2.82 ± 1.52                           | 5.90 ± 4.76                           | <0.001                            | 1252 (0.983 - 1.595)                 | 0.06    |
| Arterial Hypertension<br>Absent vs. Present | 65/11                                 | 55/15                                 | 0.27                              | -                                    |         |
| Diabetes<br>Absent vs. Present              | 74/2                                  | 52/18                                 | <0.001                            | -                                    |         |
| <b>Histology</b>                            |                                       |                                       |                                   |                                      |         |
| NAS score 0-2/3-4/5-8                       | 11/23/42                              | 3/17/50                               | 0.001                             | 0.834 (0.594- 1.172)                 | 0.29    |
| Lobular inflammation 0/1/2/3                | 8/42/25/1                             | 2/29/35/4                             | 0.002                             | -                                    |         |
| Steatosis (continuous variable)             | 41.7 ± 27.0                           | 49.4 ± 25.6                           | 0.08                              | -                                    |         |
| Steatosis grade 0/1/2/3                     | 0/33/21/22                            | 0/22/21/27                            | 0.12                              | -                                    |         |
| Hepatocellular ballooning 0/1/2             | 9/30/37                               | 4/23/43                               | 0.08                              | -                                    |         |
| Stage of Fibrosis 0/1/2/3/4                 | 26/29/18/3/0                          | 12/11/17/19/11                        | <0.001                            | 2.175 (1.359 - 3.481)                | 0.001   |

Data are given as mean ± SD or as number of cases (%). yrs indicates years; HDL, high-density lipoprotein; HOMA, homeostasis model assessment; and NAS, non-alcoholic steatohepatitis activity score.

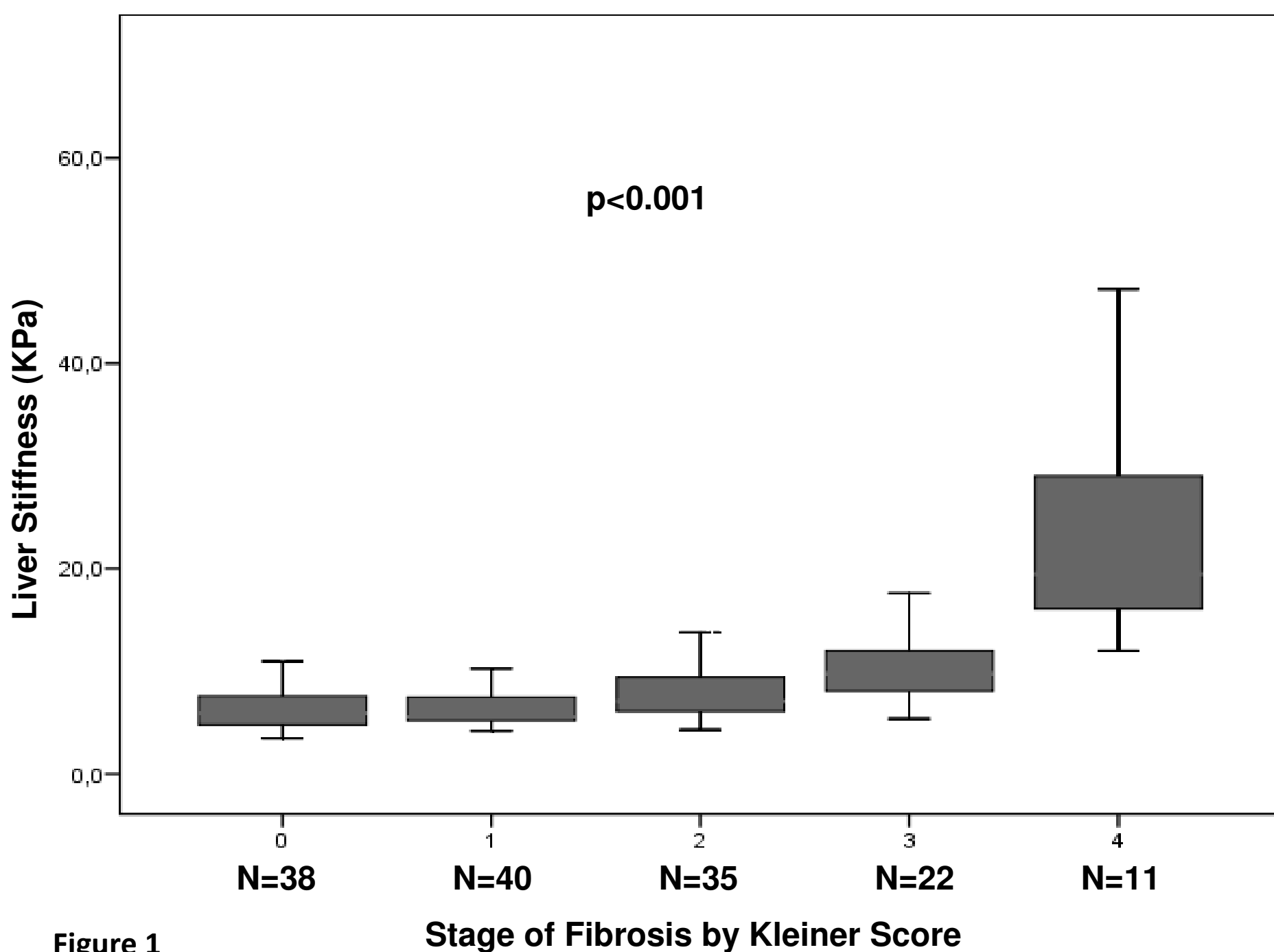


Figure 1

Stage of Fibrosis by Kleiner Score



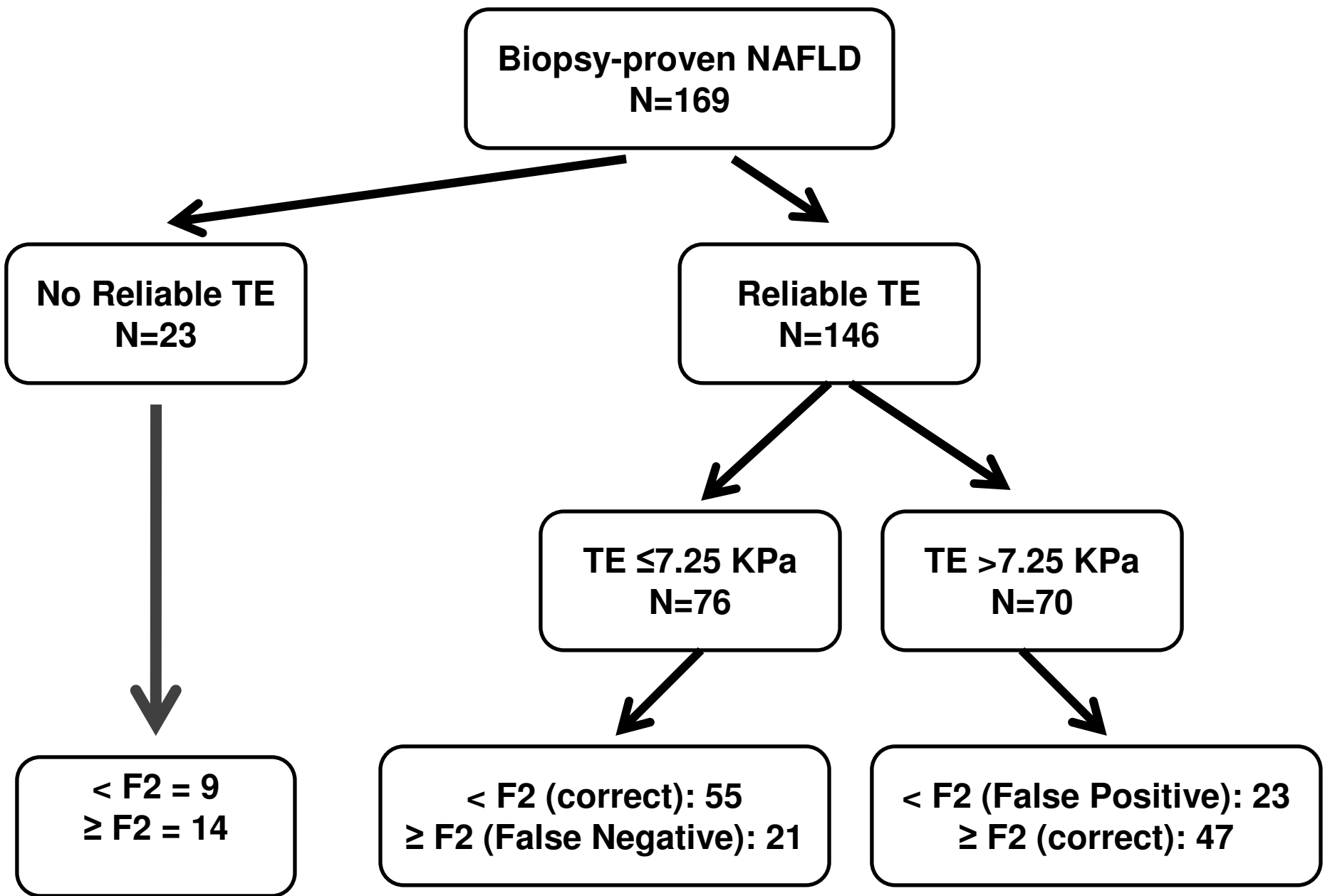


Figure 2A

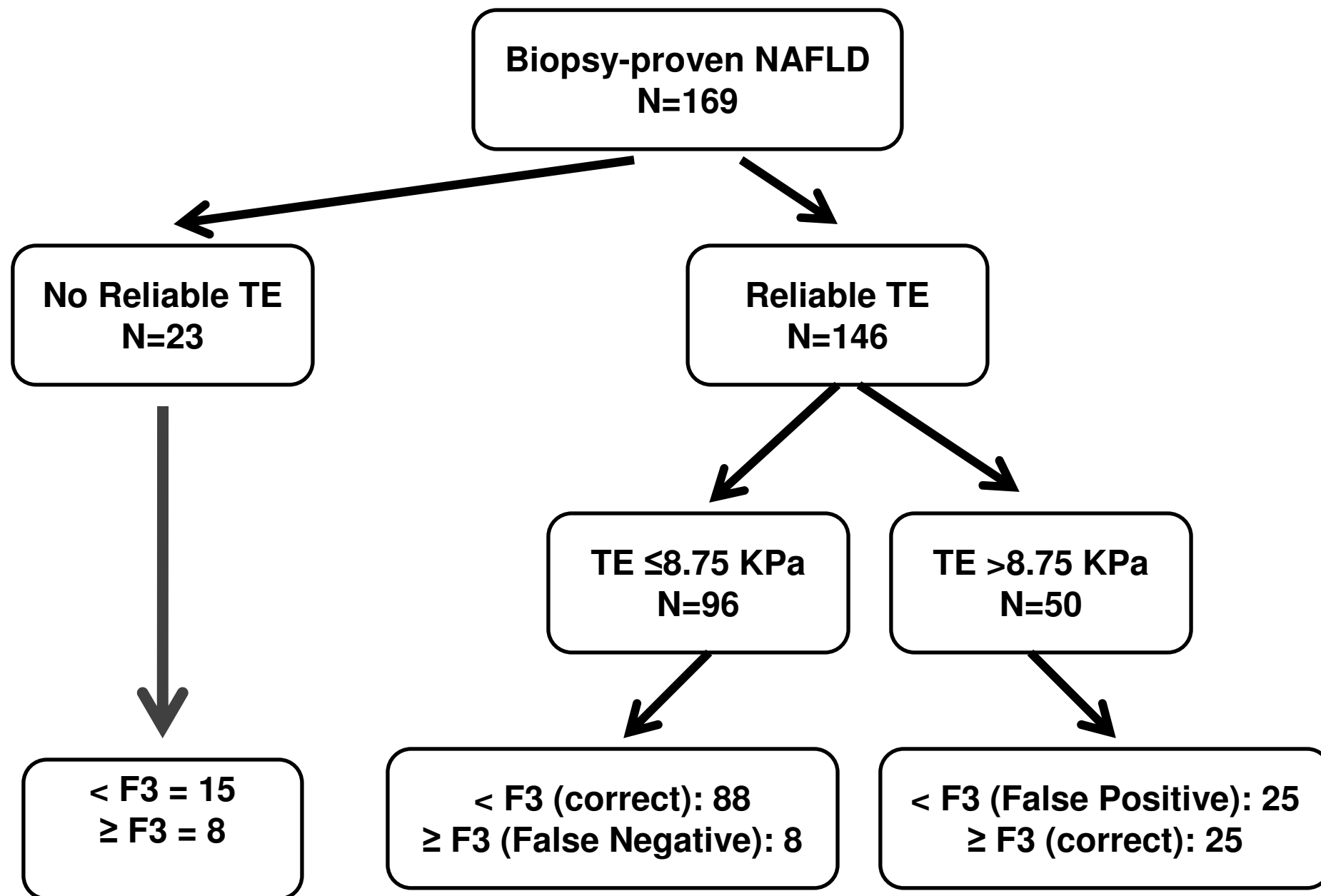
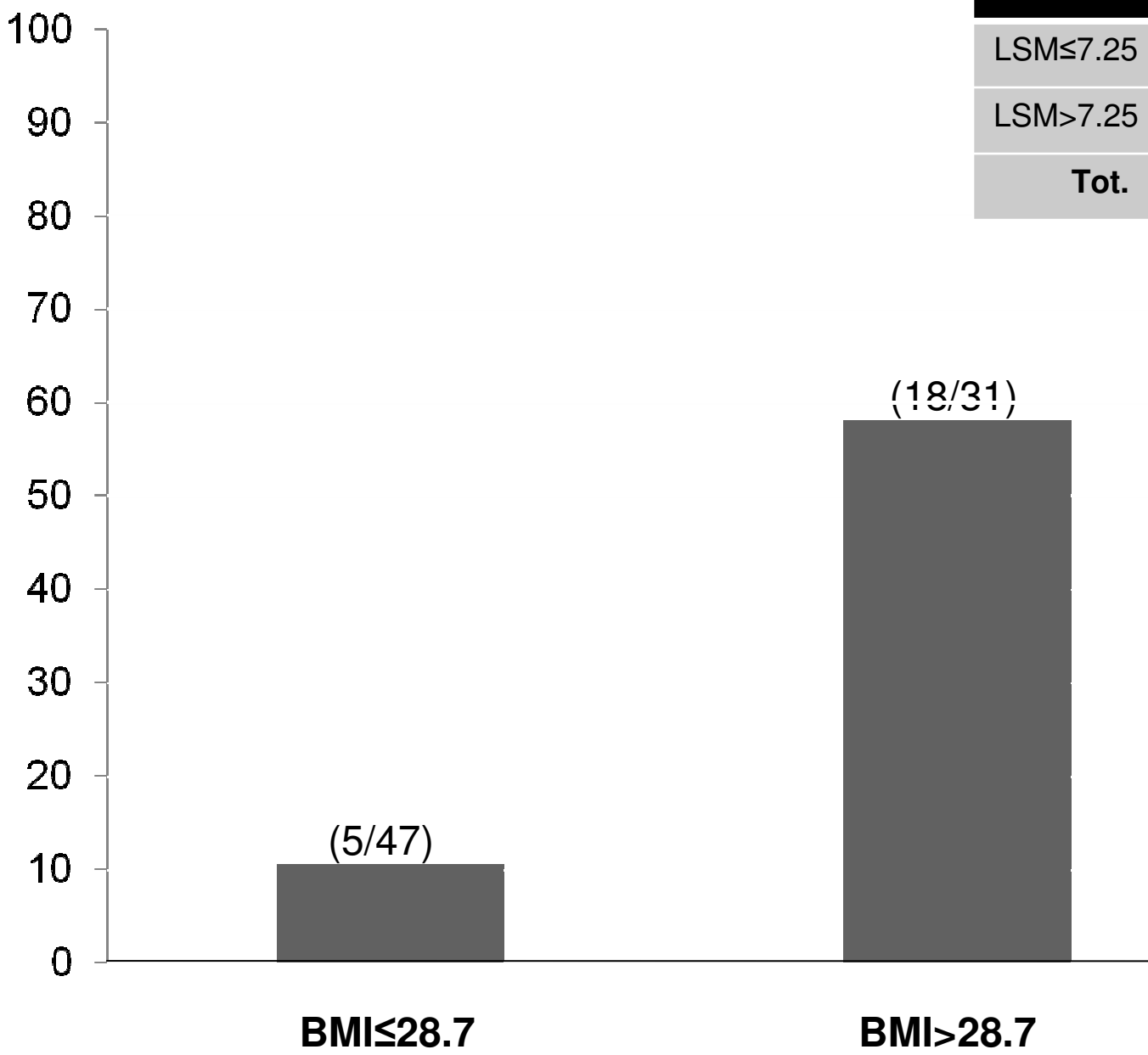


Figure 2B

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**% of false positive**



|                | <b>F0-F1</b> |
|----------------|--------------|
| LSM ≤ 7.25 KPa | 55           |
| LSM > 7.25 KPa | <b>23 FP</b> |
| <b>Tot.</b>    | <b>78</b>    |

**Figure 3A**

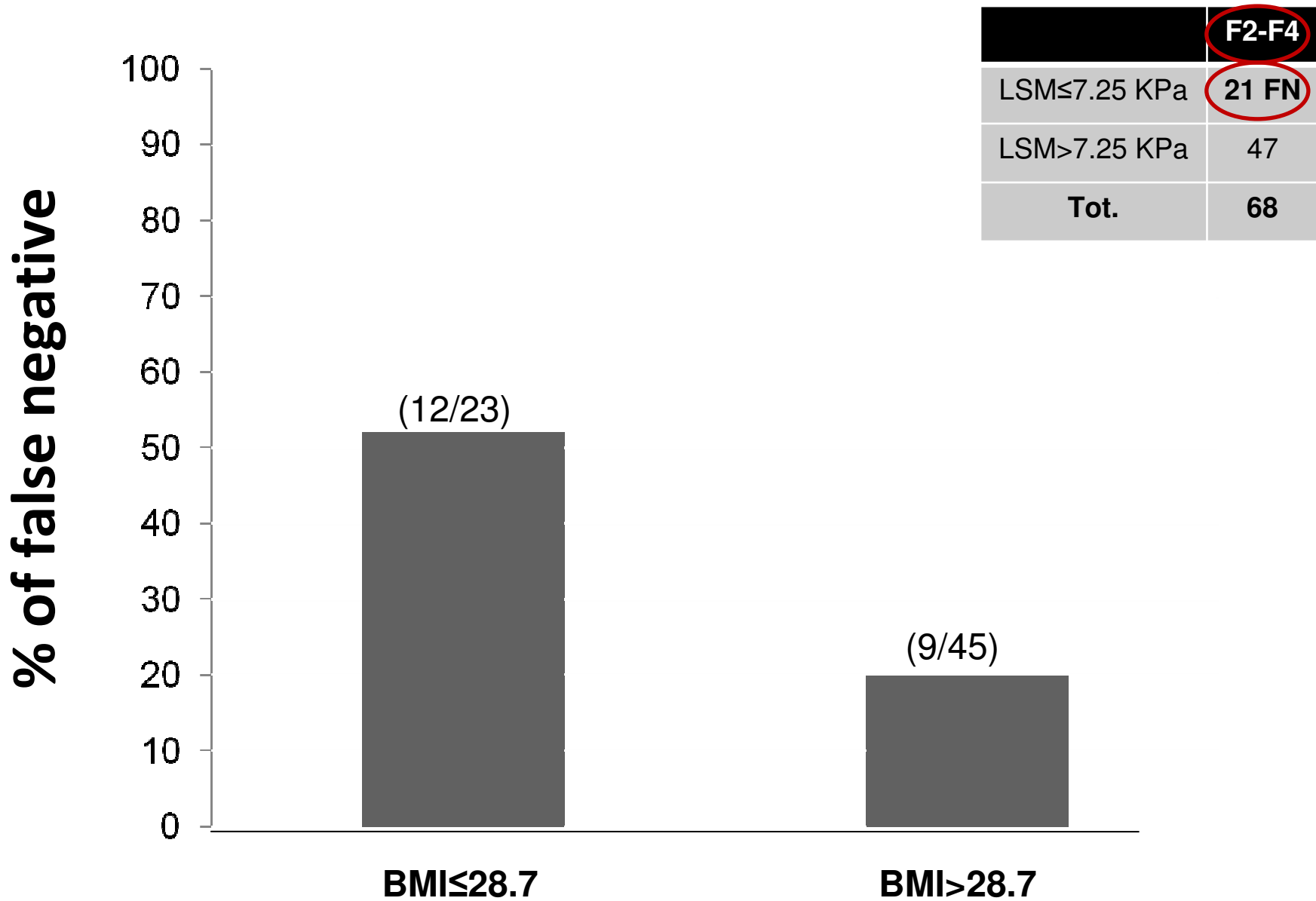
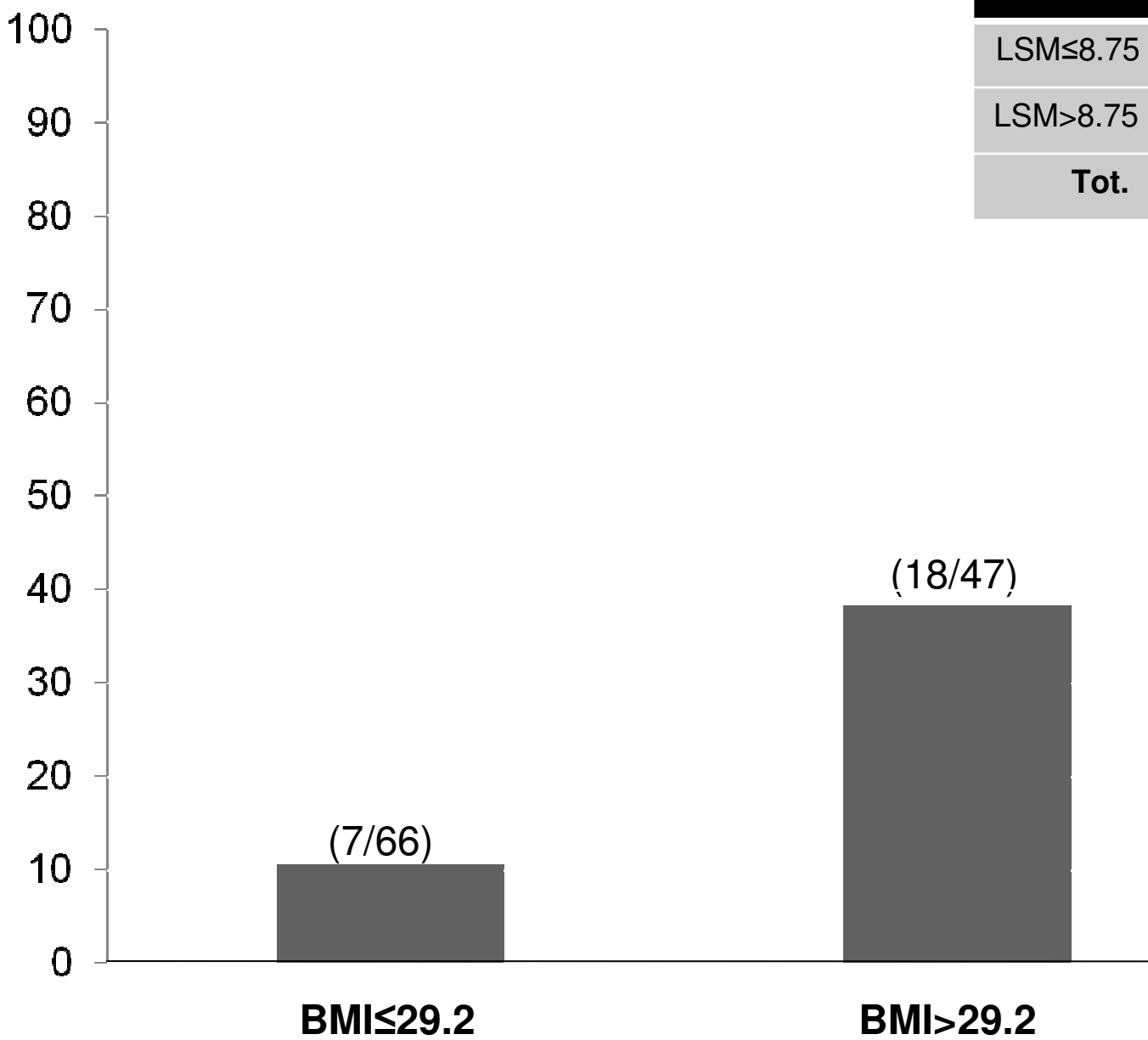


Figure 3B

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**% of false positive**

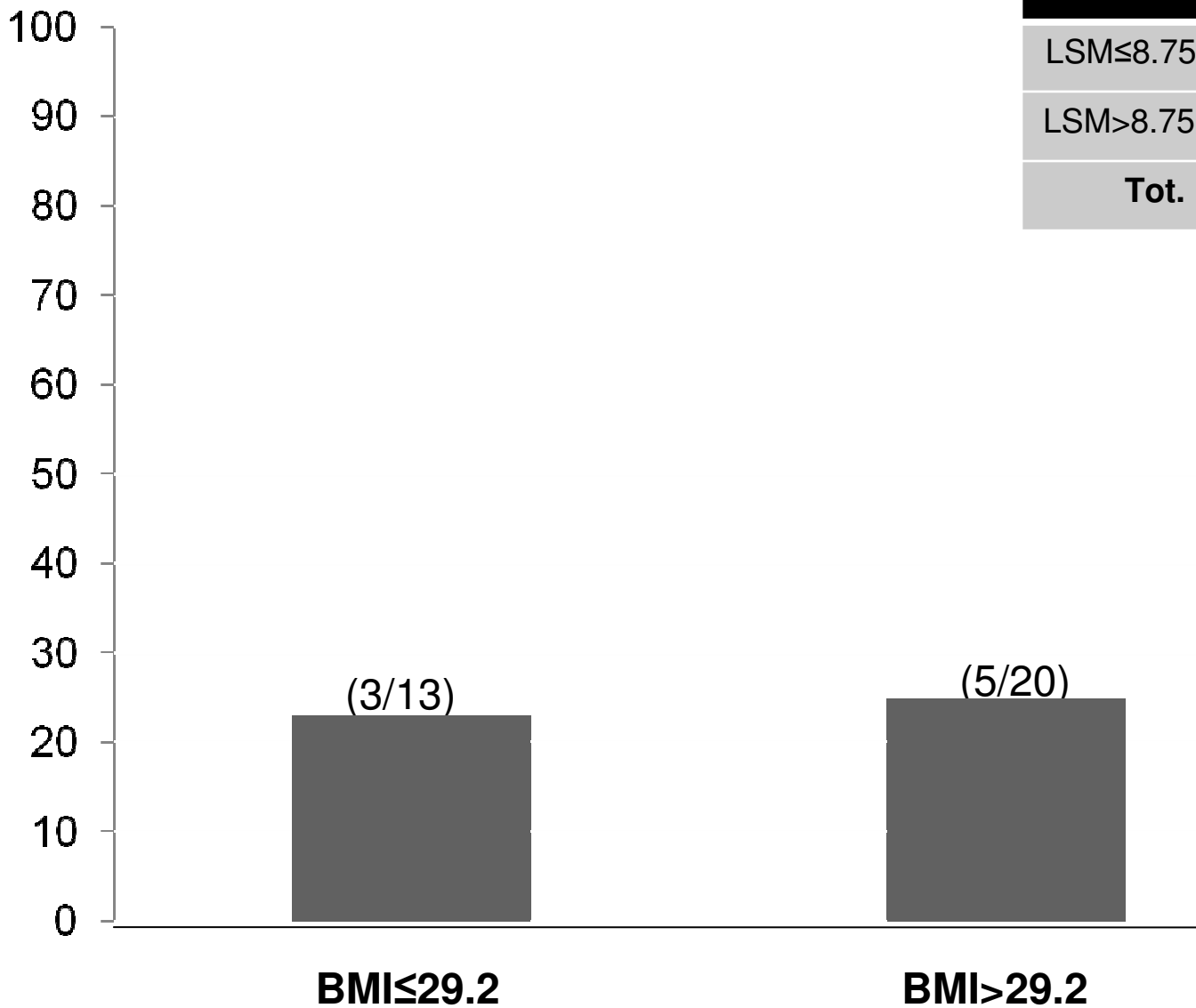


|                | <b>F0-F2</b> |
|----------------|--------------|
| LSM ≤ 8.75 Kpa | 88           |
| LSM > 8.75 KPa | <b>25 FP</b> |
| <b>Tot.</b>    | <b>113</b>   |

**Figure 4A**

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**% of false negative**



|                | <b>F3-F4</b> |
|----------------|--------------|
| LSM ≤ 8.75 Kpa | <b>8 FN</b>  |
| LSM > 8.75 KPa | 25           |
| <b>Tot.</b>    | <b>33</b>    |

**Figure 4B**

**Supporting document 1. Univariate and multivariate analysis of risk factors associated with severe fibrosis (F3-F4) in 146 patients with non-alcoholic fatty liver disease.**

| Variable                                 | No Severe Fibrosis<br>N=113 | Severe Fibrosis<br>n=33 | Univariate<br>Analysis<br><i>p</i> value | Multivariate Analysis<br>OR (95% CI) | <i>p</i> value |
|--|-----------------------------|-------------------------|--|--------------------------------------|----------------|
| Mean age – yrs                           | 41.4 ± 12.1                 | 53.5 ± 12.8             | <0.001                                   | 1.042 (0.989 – 1.099)                | 0.12           |
| Gender<br>male/ female                   | 87/26                       | 17/16                   | 0.004                                    | 1.791 (0.514 – 6.241)                | 0.36           |
| Mean body mass index – kg/m <sup>2</sup> | 28.8 ± 4.3                  | 30.0 ± 2.9              | 0.13                                     | -                                    |                |
| Waist circumference- cm                  | 97.6 ± 11.8                 | 99.2 ± 12.9             | 0.52                                     | -                                    |                |
| Alanine aminotransferase – IU            | 82.9 ± 60.7                 | 74.3 ± 46.8             | 0.45                                     | -                                    |                |
| Platelet count- 10 <sup>3</sup> mmc      | 223.1 ± 57.7                | 197.9 ± 74.4            | 0.04                                     | 1.001 (0.991 – 1.012)                | 0.78           |
| γ-glutamyl transferase – IU              | 103.7 ± 133.5               | 139.1 ± 190.1           | 0.22                                     | -                                    |                |
| Ferritin - ng/mL                         | 277.1 ± 264.9               | 261.7 ± 291.2           | 0.78                                     | -                                    |                |
| Cholesterol – mg/dL                      | 204.9 ± 49.2                | 199.0 ± 46.3            | 0.53                                     | -                                    |                |
| HDL cholesterol – mg/dL                  | 49.2 ± 17.6                 | 44.5 ± 12.1             | 0.15                                     | -                                    |                |
| Triglycerides – mg/dL                    | 147.6 ± 80.2                | 167.5 ± 83.9            | 0.21                                     | -                                    |                |
| Blood glucose – mg/dL                    | 93.8 ± 29.1                 | 110.8 ± 36.2            | 0.006                                    | -                                    |                |
| Insulin – μU/mL                          | 14.6 ± 8.4                  | 25.0 ± 14.1             | <0.001                                   | -                                    |                |
| HOMA-score                               | 3.45 ± 2.69                 | 7.20 ± 5.35             | <0.001                                   | 1.132 (0.968 – 1.324)                | 0.12           |
| Diabetes                                 | 100/13                      | 26/7                    | 0.15                                     | -                                    |                |
| Arterial Hypertension                    | 96/17                       | 24/9                    | 0.10                                     | -                                    |                |
| Liver Stiffness - KPa                    | 7.0 ± 2.6                   | 15.6 ± 11.9             | <0.001                                   | 1.280 (1.094 - 1.497)                | 0.002          |
| <b>Histology</b>                         |                             |                         |  |                                      |                |
| NAS score 0-2/3-4/5-8                    | 14/34/65                    | 0/6/27                  | 0.004                                    | -                                    |                |
| Lobular inflammation 0/1/2/3             | 10/63/38/2                  | 0/8/22/3                | <0.001                                   | 4.060 (1.478 – 11.149)               | 0.007          |
| Steatosis (continuous variable)          | 45.3 ± 27.3                 | 45.3 ± 24.2             | 0.94                                     | -                                    |                |
| Steatosis grade 0/1/2/3                  | 0/42/32/39                  | 0/13/10/10              | 0.70                                     | -                                    |                |
| Hepatocellular ballooning 0/1/2          | 13/55/55                    | 0/8/25                  | 0.003                                    | 1.668 (0.537 – 5.185)                | 0.37           |

Data are given as mean ± SD or as number of cases (%). yrs indicates years; HDL, high-density lipoprotein; HOMA, homeostasis model assessment; and NAS, non-alcoholic steatohepatitis activity score.

**Supporting document 2. Univariate and multivariate analysis of factors associated with liver stiffness >7.25 Kpa for significant fibrosis in 146 patients with non-alcoholic fatty liver disease, replacing in the model BMI with waist circumference.**

| Variable                                  | Liver Stiffness<br>≤ 7.25 Kpa<br>n=76 | Liver Stiffness<br>> 7.25 Kpa<br>n=70 | Univariate Analysis<br>p value | Multivariate Analysis<br>OR (95% CI)<br>value | P     |
|---|---------------------------------------|---------------------------------------|--------------------------------|---|-------|
| Age – yrs                                 | 41.3 ± 10.9                           | 47.2 ± 14.8                           | 0.007                          | 1.006 (0.965 – 1.049)                         | 0.76  |
| <b>Gender</b>                             |                                       |                                       |                                |   |       |
| Male vs. Female                           | 61/15                                 | 43/27                                 | 0.01                           | 3.293 (0.926 – 11.716)                        | 0.47  |
| <b>Body Mass Index – Kg/m<sup>2</sup></b> | 27.3 ± 3.4                            | 31.1 ± 3.8                            | <0.001                         | -   |       |
| <b>Waist Circumference- cm</b>            | 94.0 ± 9.8                            | 102.6 ± 12.8                          | <0.001                         | 1.066 (1.019 – 1.116)                         | 0.006 |
| Alanine aminotransferase – IU             | 85.5 ± 66.1                           | 76.0 ± 47.1                           | 0.32                           | -   |       |
| Platelet count- 10 <sup>3</sup> mmc       | 222.5 ± 53.1                          | 211.9 ± 71.2                          | 0.30                           | -   |       |
| γ glutamyl transferase – IU               | 109.0 ± 131.5                         | 114.7 ± 165.3                         | 0.82                           | -   |       |
| Ferritin – ng/mL                          | 256.2 ± 231.2                         | 293.7 ± 308.8                         | 0.40                           | -   |       |
| Cholesterol – mg/Dl                       | 205.8 ± 47.7                          | 201.1 ± 49.4                          | 0.56                           | -   |       |
| HDL Cholesterol – mg/Dl                   | 50.7 ± 19.1                           | 45.3 ± 12.9                           | 0.05                           | 0.978 (0.939 - 1.020)                         | 0.29  |
| Triglycerides – mg/Dl                     | 143.2 ± 79.1                          | 161.6 ± 82.3                          | 0.17                           | -   |       |
| Blood glucose – mg/dL                     | 86.8 ± 12.0                           | 109.4 ± 40.7                          | <0.001                         | -   |       |
| Insulin – μU/mL                           | 13.0 ± 6.7                            | 21.2 ± 12.8                           | <0.001                         | -   |       |
| HOMA-score                                | 2.82 ± 1.52                           | 5.90 ± 4.76                           | <0.001                         | 1.258 (0.981 - 1.596)                         | 0.06  |
| <b>Arterial Hypertension</b>              |                                       |                                       |                                |   |       |
| Absent vs. Present                        | 65/11                                 | 55/15                                 | 0.27                           | -   |       |
| <b>Diabetes</b>                           |                                       |                                       |                                |   |       |
| Absent vs. Present                        | 74/2                                  | 52/18                                 | <0.001                         | -   |       |
| <b>Histology</b>                          |                                       |                                       |                                |   |       |
| NAS score 0-2/3-4/5-8                     | 11/23/42                              | 3/17/50                               | 0.001                          | 0.795 (0.521- 1.215)                          | 0.28  |
| Lobular inflammation 0/1/2/3              | 8/42/25/1                             | 2/29/35/4                             | 0.002                          | -   |       |
| Steatosis (continuous variable)           | 41.7 ± 27.0                           | 49.4 ± 25.6                           | 0.08                           | -   |       |
| Steatosis grade 0/1/2/3                   | 0/33/21/22                            | 0/22/21/27                            | 0.12                           | -   |       |
| Hepatocellular ballooning 0/1/2           | 9/30/37                               | 4/23/43                               | 0.08                           | -   |       |
| Stage of Fibrosis 0/1/2/3/4               | 26/29/18/3/0                          | 12/11/17/19/11                        | <0.001                         | 2.466 (1.415 - 4.300)                         | 0.001 |

Data are given as mean ± SD or as number of cases (%). yrs indicates years; HDL, high-density lipoprotein; HOMA, homeostasis model assessment; and NAS, non-alcoholic steatohepatitis activity score.



**Supporting document 3. Univariate and multivariate analysis of factors associated with liver stiffness >8.75 KPa for significant fibrosis in 146 patients with non-alcoholic fatty liver disease.**

| Variable                            | Liver Stiffness<br>≤ 8.75 Kpa<br>n=96 | Liver Stiffness<br>> 8.75 Kpa<br>n=50 | Univariate<br>Analysis<br>p value | Multivariate Analysis<br>OR (95% CI) | P value |
|-------------------------------------|---------------------------------------|---------------------------------------|-----------------------------------|--------------------------------------|---------|
| Age – yrs                           | 41.6 ± 11.6                           | 49.0 ± 14.8                           | 0.001                             | 1.012 (0.978 – 1.047)                | 0.50    |
| Gender                              |                                       |                                       |                                   |                                      |         |
| Male vs. Female                     | 76/20                                 | 28/22                                 | 0.003                             | 1.743 (0.684 - 4.443)                | 0.24    |
| Body Mass Index – Kg/m <sup>2</sup> | 28.0 ± 3.8                            | 31.2 ± 3.8                            | <0.001                            | 1.190 (1.056 - 1.340)                | 0.004   |
| Waist Circumference- cm             | 95.4 ± 10.1                           | 103.0 ± 13.8                          | 0.001                             | -                                    |         |
| Alanine aminotransferase – IU       | 84.2 ± 60.9                           | 74.5 ± 51.3                           | 0.33                              | -                                    |         |
| Platelet count- 10 <sup>3</sup> mmc | 220.3 ± 50.9                          | 212.0 ± 80.5                          | 0.45                              | -                                    |         |
| γ glutamyl transferase – IU         | 102.3 ± 122.9                         | 129.9 ± 187.6                         | 0.28                              | -                                    |         |
| Ferritin – ng/mL                    | 253.6 ± 219.0                         | 312.3 ± 347.1                         | 0.21                              | -                                    |         |
| Cholesterol – mg/Dl                 | 205.0 ± 47.7                          | 200.7 ± 50.3                          | 0.61                              | -                                    |         |
| HDL Cholesterol – mg/Dl             | 49.4 ± 17.7                           | 45.7 ± 13.9                           | 0.20                              | -                                    |         |
| Triglycerides – mg/Dl               | 142.5 ± 76.8                          | 170.2 ± 86.2                          | 0.05                              | 0.999 (0.993 - 1.005)                | 0.76    |
| Blood glucose – mg/dL               | 89.0 ± 16.8                           | 114.1 ± 44.4                          | <0.001                            | -                                    |         |
| Insulin – μU/mL                     | 14.0 ± 7.1                            | 22.7 ± 14.3                           | <0.001                            | -                                    |         |
| HOMA-score                          | 3.14 ± 1.92                           | 6.51 ± 5.27                           | <0.001                            | 1.169 (0.989 - 1.382)                | 0.06    |
| Arterial Hypertension               |                                       |                                       |                                   |                                      |         |
| Absent vs. Present                  | 82/14                                 | 38/12                                 | 0.15                              | -                                    |         |
| Diabetes                            |                                       |                                       |                                   |                                      |         |
| Absent vs. Present                  | 93/3                                  | 33/17                                 | <0.001                            | -                                    |         |
| Histology                           |                                       |                                       |                                   |                                      |         |
| NAS score 0-2/3-4/5-8               | 12/30/54                              | 2/10/38                               | 0.003                             | 0.892 (0.624- 1.277)                 | 0.53    |
| Lobular inflammation 0/1/2/3        | 8/53/34/1                             | 2/18/26/4                             | 0.006                             | -                                    |         |
| Steatosis (continuous variable)     | 43.0 ± 26.5                           | 50.0 ± 26.3                           | 0.13                              | -                                    |         |
| Steatosis grade 0/1/2/3             | 0/39/28/29                            | 0/16/14/20                            | 0.21                              | -                                    |         |
| Hepatocellular ballooning 0/1/2     | 11/37/48                              | 2/16/32                               | 0.06                              | -                                    |         |
| Stage of Fibrosis 0/1/2/3/4         | 31/35/22/8/0                          | 7/5/13/14/11                          | <0.001                            | 2.315 (1.459 - 3.675)                | <0.001  |

Data are given as mean ± SD or as number of cases (%). yrs indicates years; HDL, high-density lipoprotein; HOMA, homeostasis model assessment; and NAS, non-alcoholic steatohepatitis activity score.

**Supporting document 4. Univariate and multivariate analysis of factors associated with liver stiffness >8.75 KPa for significant fibrosis in 146 patients with non-alcoholic fatty liver disease, replacing in the model BMI with waist circumference.**

| Variable                            | Liver Stiffness<br>≤ 8.75 Kpa<br>n=96 | Liver Stiffness<br>> 8.75 Kpa<br>n=50 | Univariate<br>Analysis<br>p value | Multivariate Analysis<br>OR (95% CI) | P value |
|-------------------------------------|---------------------------------------|---------------------------------------|-----------------------------------|--------------------------------------|---------|
| Age – yrs                           | 41.6 ± 11.6                           | 49.0 ± 14.8                           | 0.001                             | 1.010 (0.969 – 1.054)                | 0.63    |
| Gender                              |                                       |                                       |                                   |                                      |         |
| Male vs. Female                     | 76/20                                 | 28/22                                 | 0.003                             | 3.837 (0.974 - 12.524)               | 0.06    |
| Body Mass Index – Kg/m <sup>2</sup> | 28.0 ± 3.8                            | 31.2 ± 3.8                            | <0.001                            | -                                    |         |
| Waist Circumference- cm             | 95.4 ± 10.1                           | 103.0 ± 13.8                          | 0.001                             | 1.068 (1.015 - 1.123)                | 0.01    |
| Alanine aminotransferase – IU       | 84.2 ± 60.9                           | 74.5 ± 51.3                           | 0.33                              | -                                    |         |
| Platelet count- 10 <sup>3</sup> mmc | 220.3 ± 50.9                          | 212.0 ± 80.5                          | 0.45                              | -                                    |         |
| γ glutamyl transferase – IU         | 102.3 ± 122.9                         | 129.9 ± 187.6                         | 0.28                              | -                                    |         |
| Ferritin – ng/mL                    | 253.6 ± 219.0                         | 312.3 ± 347.1                         | 0.21                              | -                                    |         |
| Cholesterol – mg/Dl                 | 205.0 ± 47.7                          | 200.7 ± 50.3                          | 0.61                              | -                                    |         |
| HDL Cholesterol – mg/Dl             | 49.4 ± 17.7                           | 45.7 ± 13.9                           | 0.20                              | -                                    |         |
| Triglycerides – mg/Dl               | 142.5 ± 76.8                          | 170.2 ± 86.2                          | 0.05                              | 0.999 (0.992 - 1.007)                | 0.85    |
| Blood glucose – mg/dL               | 89.0 ± 16.8                           | 114.1 ± 44.4                          | <0.001                            | -                                    |         |
| Insulin – μU/mL                     | 14.0 ± 7.1                            | 22.7 ± 14.3                           | <0.001                            | -                                    |         |
| HOMA-score                          | 3.14 ± 1.92                           | 6.51 ± 5.27                           | <0.001                            | 1.173 (0.931 - 1.398)                | 0.06    |
| Arterial Hypertension               |                                       |                                       |                                   |                                      |         |
| Absent vs. Present                  | 82/14                                 | 38/12                                 | 0.15                              | -                                    |         |
| Diabetes                            |                                       |                                       |                                   |                                      |         |
| Absent vs. Present                  | 93/3                                  | 33/17                                 | <0.001                            | -                                    |         |
| Histology                           |                                       |                                       |                                   |                                      |         |
| NAS score 0-2/3-4/5-8               | 12/30/54                              | 2/10/38                               | 0.003                             | 0.913 (0.578- 1.443)                 | 0.69    |
| Lobular inflammation 0/1/2/3        | 8/53/34/1                             | 2/18/26/4                             | 0.006                             | -                                    |         |
| Steatosis (continuous variable)     | 43.0 ± 26.5                           | 50.0 ± 26.3                           | 0.13                              | -                                    |         |
| Steatosis grade 0/1/2/3             | 0/39/28/29                            | 0/16/14/20                            | 0.21                              | -                                    |         |
| Hepatocellular ballooning 0/1/2     | 11/37/48                              | 2/16/32                               | 0.06                              | -                                    |         |
| Stage of Fibrosis 0/1/2/3/4         | 31/35/22/8/0                          | 7/5/13/14/11                          | <0.001                            | 3.001 (1.697 - 5.305)                | <0.001  |

Data are given as mean ± SD or as number of cases (%). yrs indicates years; HDL, high-density lipoprotein; HOMA, homeostasis model assessment; and NAS, non-alcoholic steatohepatitis activity score.