Performance of liver stiffness measurements obtained with FibroScan is affected by glucose metabolism in patients with nonalcoholic fatty liver disease

Xinyu Yang
Department of Endocrinology and Metabolism, Zhongshan Hospital, Fudan University, Shanghai, China

Xinxia Chang
Department of Endocrinology and Metabolism, Zhongshan Hospital, Fudan University, Shanghai, China

Shengdi Wu
Zhongshan Hospital Fudan University

Xiaoyang Sun
Zhongshan Hospital Fudan University

Xiaopeng Zhu
Zhongshan Hospital Fudan University

Liu Wang
Department of Endocrinology and Metabolism, Zhongshan Hospital Fudan University

Yushan Xu
Zhongshan Hospital Fudan University

Xiuzhong Yao
Zhongshan Hospital Fudan University

Shengxiang Rao
Zhongshan Hospital Fudan University

Xiqi Hu
Shanghai Medical University: Fudan University

Mingfeng Xia
Zhongshan Hospital Fudan University

Hua Bian
Zhongshan Hospital Fudan University

Hong-Mei Yan (yan.hongmei@zs-hospital.sh.cn)
Zhongshan Hospital Fudan University  https://orcid.org/0000-0001-7341-4368

Xin Gao
Zhongshan Hospital Fudan University
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Abstract

**Background:** The performance of liver stiffness measurements (LSMs) obtained using FibroScan can be affected by several factors, and cut-off values are different for fibrosis caused by various aetiologies. The study aims to evaluate the diagnostic accuracy of LSM in nonalcoholic fatty liver disease (NAFLD) patients with abnormal glucose metabolism and investigate whether the LSM value would be affected by metabolic indicators.

**Methods:** The study involved 91 NAFLD patients with abnormal glucose metabolism who underwent liver biopsy. The diagnostic accuracy of LSM value was evaluated by the receiver operator characteristic (ROC) curves, with the biopsy results taken as the gold standard. Multivariate linear regression and subgroup analysis were performed to determine the correlated indicators.

**Results:** The areas under the ROC curves (AUROCs) of LSM values for detecting fibrosis stage ≥1, 2, 3 and 4 were 0.793 (95% confidence interval [CI]: 0.695-0.871), 0.764 (95% CI: 0.663-0.846), 0.837 (95% CI: 0.744-0.906) and 0.902 (95% CI: 0.822-0.955), with cut-off values of 6.3, 7.6, 8.3 and 13.8 kPa, respectively. Multivariate linear regression demonstrated that haemoglobin A1c (HbA1c, $\beta = 0.200$, $P = 0.038$) and aspartate aminotransferase (AST, $\beta = 0.200$, $P = 0.044$) were independently associated with the LSM value after adjustment for fibrosis stage, ballooning and inflammation grade from liver biopsy. Subgroup analysis demonstrated that LSM values were slightly higher in patients with HbA1c ≥7% than in those with HbA1c <7% and in patients with body mass index (BMI) ≥30 kg/m$^2$ than in those with BMI <30 kg/m$^2$.

**Conclusions:** FibroScan was valuable for the evaluation of liver fibrosis in NAFLD patients with abnormal glucose metabolism. FibroScan is recommended to evaluate severe fibrosis, especially to exclude advanced fibrosis. Glucose metabolism state may affect LSM values.

Introduction

Nonalcoholic fatty liver disease (NAFLD) has become the predominant cause of chronic liver injury worldwide and refers to the presence of ≥5% hepatic steatosis (HS) without other competing aetiologies, including chronic viral hepatitis, excessive alcohol consumption, the use of steatogenic medication or hereditary disorders [1]. Its spectrum ranges from nonalcoholic fatty liver (NAFL) and nonalcoholic steatohepatitis (NASH) to cirrhosis or even carcinoma, and it is associated with features of metabolic syndrome, such as hypertension, insulin resistance, diabetes mellitus (DM) and dyslipidaemia. NAFLD increases risks of cardiovascular disease and accelerates the progression of underlying disease, leading to severe consequences [2-4]. NAFLD patients with DM are prone to develop NASH, liver fibrosis and cirrhosis, and even liver cancer. The overall prevalence of NAFLD among patients with type 2 diabetes mellitus (T2DM) is 55%, more than 2-fold higher than that in the general population, with a very high rate of NASH [5-7]. In the course of NAFLD, liver fibrosis, an important predictor of adverse prognosis, is the most relevant target for early diagnosis and treatment, as it has been associated with further
deterioration of cirrhosis and increased overall mortality [8, 9]. In the authors’ recent study, fibrosis occurred in up to 50% of patients with both NAFLD and T2DM [10]. Considering the large number of patients with T2DM, the burden of NAFLD management appears enormous. Although effective therapies for NAFLD have not been established, early intervention can significantly improve its poor prognosis. Therefore, the accurate and early detection of NAFLD in patients with abnormal glucose metabolism, especially the determination of fibrosis stage of liver fibrosis, is crucial [8, 11, 12].

Liver biopsy, an invasive procedure, has been recommended as the gold standard for the diagnosis and classification of NAFLD, but the limitations of possible bleeding risks and sampling errors make it unsuitable for screening and frequent monitoring [13-15]. Therefore, noninvasive alternatives to liver biopsy have been investigated, such as serum biomarkers, clinical scoring systems and imaging tests, including ultrasonography, proton magnetic resonance spectroscopy (1H-MRS), magnetic resonance imaging-derived proton density fat fraction (MRI-PDFF), FibroScan and magnetic resonance elastography (MRE) [16, 17]. Among them, FibroScan (transient elastography; EchoSens, Paris, France) is recommended by the 2018 NAFLD guidance because of its convenience, clinical accessibility, low cost and simultaneous measurement of fibrosis and steatosis [16]. Liver stiffness measurement (LSM) obtained using FibroScan is one parameter for the diagnosis and quantification of liver fibrosis by measuring mechanical or ultrasound shear wave propagation through the hepatic parenchyma [18, 19].

Several studies have assessed the diagnostic performance of FibroScan, most of which targeted patients with chronic hepatitis B [20, 21], while others focused on NAFLD [18, 22, 23]. Transient elastography expert consensus pointed out that there are differences in the cut-off values in patients with liver fibrosis caused by various aetiologies, consisting of hepatitis B, hepatitis C and NAFLD. Factors such as liver inflammation activity manifested by alanine aminotransferase (ALT) or increased bilirubin levels, excessive alcohol intake and eating may lead to an increase in LSM values [24]. In view of the promoting effect of abnormal glucose metabolism on liver disease, NAFLD patients with abnormal glucose metabolism may have different specific cut-off values than general NAFLD patients. However, to date, there have been no studies focusing on these populations and the lack of cut-off values.

The study aims to evaluate the diagnostic performance of FibroScan and obtain cut-off values in NAFLD patients with abnormal glucose metabolism and to investigate whether metabolic indicators would affect the measurement of FibroScan to provide clinical advice for the application of FibroScan in the diagnosis and evaluation of NAFLD patients.

**Material And Methods**

**Design and subjects**

This cross-sectional study included 91 NAFLD patients evaluated in Zhongshan Hospital, Fudan University, between July 2015 and December 2019, 89 of whom underwent liver biopsy, while 2 others had cirrhosis based on ultrasound. The time interval between FibroScan examination and biopsy was <2
weeks. Patients with a history of excessive alcoholic consumption (>20 g for men or >10 g for women/day), chronic viral hepatitis, drug use and any other causes associated with liver injury were excluded. The entire process of the study was conducted according to the ethical guidelines of the Declaration of Helsinki and was approved by the Human Research Ethics Committee of Zhongshan Hospital Clinic (B2013-132). All patients have signed an informed consent before study.

Liver biopsy and histopathologic evaluations

Liver biopsy samples were obtained using a 16-gauge needle from the right liver lobe of NAFLD patients under ultrasound guidance. Two specimens were obtained from each person to ensure a sample size sufficient for analysis and to reduce error. All biopsy specimens were evaluated by two experienced pathologists blinded to the clinical and biological data. Histopathological findings were reported in accordance with the guidelines of the Pathology Working Group of NASH Clinical Research Network of the National Institutes of Health [25]. The NAFLD activity score (NAS) consists of three parts: 1) hepatic steatosis grade 0-3, 2) lobular inflammation grade 0-3, 3) hepatocyte ballooning grade 0-2. Fibrosis was staged from 0 to 4. The classification criteria are described in Table 1. NASH was defined as NAS >4 or NAS =4 with the manifestation of steatosis, inflammation, and ballooning at the same time [25, 26].

LSM measurement

LSM measurement was performed with FibroScan using the M probe, as in most previous studies [18, 23]. Details of measurement are described in several previous studies, performed with the same machine by the same experienced operator blinded to other noninvasive methods and biopsy results [23, 27]. The examination duration was less than five minutes. Ten valid measurements were obtained from each patient and then the success rate, the ratio of the successful measurement times over the total times, was calculated. The result was considered reliable only when the success rate was ≥60% and the interquartile range (IQR)/median was ≤ 30%. The median value was kept as a representative result. Liver stiffness measurement results are expressed in kilopascals (kPa).

Basic characteristics collection

The medical history of each patient was collected, including general physical characteristics, history of chronic diseases and history of medication. Hypertension was diagnosed according to criteria [28]. Routine serological tests were performed upon admittance. Fasting blood samples were collected locally and then shipped to the clinical laboratory of Zhongshan Hospital for assessment of blood glucose, lipid profiles and other blood biochemical parameters. Abnormal glucose metabolism was defined as fasting glucose ≥ 5.6 mmol/L or 2 h glucose ≥ 7.8 mmol/L according to 2003 guidelines of the American Diabetes Association [29, 30].

Statistical analysis

Continuous variables with a normal distribution are summarized as the mean ± SD, while those without a normal distribution are described as the median (interquartile range). Categorical variables were
summarized as frequencies and percentages. SPSS software (version 23.0) was used for data analysis. The graphs were generated with GraphPad (version 8.4) and MedCalc (version 19.1). The diagnostic accuracy was evaluated by the receiver operator characteristic (ROC) curves. The area under the ROC curve (AUROC) and the cut-off value optimized with Youden's index for each degree were calculated, with sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) obtained. The unpaired t test and Kruskal-Wallis test with Dunn's multiple correction were used for univariate comparisons between groups. The independent correlation was analysed using multivariate linear regression analysis. Spearman's rank correlation coefficient was used to assess the correlation between the liver histopathologic degree and FibroScan results in patients who underwent biopsy. A $P$ value < 0.05 was considered statistically significant.

Results

Patient characteristics

In this study, a total of 91 patients with NAFLD were involved. All patients received blood biochemical examination. LSM and CAP values obtained using FibroScan were attempted in all patients. The physical, clinical, serological, and histologic characteristics are detailed in Table 1.

Evaluation of diagnostic accuracy of FibroScan on liver fibrosis in patients with NAFLD

The LSM was used to assess the stage of liver fibrosis measured by FibroScan in patients with NAFLD. The median LSM values for stages 0, 1, 2, 3, and 4 were 6.15, 6.60, 7.70, 10.50 and 14.60 kPa, respectively. The results are shown in Fig. 1a and revealed significant stepwise increases in the LSM with increasing histologic severity of hepatic fibrosis. To investigate the diagnostic accuracy of FibroScan, the ROC curves were differentiated between liver fibrosis at stage 0 vs 1-4, 0-1 vs 2-4, 0-2 vs 3-4, and 0-3 vs 4, as shown in Fig. 1b. The AUROCs in diagnosing liver fibrosis stages 1, 2, 3 and 4 were 0.793 (95% confidence interval [CI]: 0.695-0.871), 0.764 (95% CI: 0.663-0.846), 0.837 (95% CI: 0.744-0.906) and 0.902 (95% CI: 0.822-0.955), respectively. Optimized with Youden's index, the cut-off values of LSM were 6.3, 7.6, 8.3, and 13.8 kPa in detecting stage 1, 2, 3, and 4 liver fibrosis in NAFLD patients with abnormal glucose metabolism. The results are detailed in Table 2. It seemed that the diagnostic accuracy of LSM improved as the histologic severity of hepatic fibrosis increased. The diagnostic AUROC at stage 4 even reached up to 0.902, indicating that FibroScan is an ideal machine to evaluate the severity of liver fibrosis, especially to more severe degrees. In addition, the cut-off value for each stage with sensitivity $\geq 90\%$ or specificity $\geq 90\%$ was added to Additional file 1-Table 1.

Influence of metabolic indicators on FibroScan in detecting liver fibrosis

Multivariate linear regression analysis was conducted to investigate whether metabolic state would affect the LSM value. Metabolic indicators, including age, body mass index (BMI), hypertension, fasting glucose, 2 h glucose, haemoglobin A1c (HbA1c), triglyceride (TG), total cholesterol (TC), LDL cholesterol, HDL cholesterol, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), were included
as independent variables, and the LSM value was included as the dependent variable in the stepwise backward regression analysis after adjustment for the histopathological degree of liver fibrosis, ballooning and inflammation, as shown in Table 3. The results demonstrated that HbA1c ($\beta = 0.200, P = 0.038$) and AST ($\beta = 0.200, P = 0.044$) were independently correlated with the LSM value after adjustment for the liver histopathological degree. BMI ($\beta = 0.164, P = 0.092$) also entered the regression model, while no statistical significance was obtained. To further investigate the influence of metabolic indicators on FibroScan measurements, subgroup analysis of LSM values was conducted grouped by HbA1c <7% or HbA1c $\geq$ 7% and BMI <30 kg/m$^2$ or BMI $\geq$ 30 kg/m$^2$. The fibrosis stage was divided into stage 0-1 and stage 2-4 (significant fibrosis). The violin plots in Fig. 2a demonstrated a trend that LSM values were higher for patients with HbA1c $\geq$ 7% than for patients with HbA1c <7%, and statistical significance was detected in patients without significant fibrosis. Given that poorly controlled glucose aggravates liver inflammatory activity and inflammation may influence FibroScan measurements, the LSM values between subgroups were matched for histological inflammation and ballooning degree. Interestingly, the increasing trend and statistical significance remained when matching for inflammation or ballooning grade, as shown in Additional file 2- Fig. 1a and Fig. 1b. Subgroup analysis of BMI revealed a similar trend, and statistical significance was detected in patients with significant fibrosis, as shown in Fig. 2b. In view of the results above, an LSM cut-off value in detecting liver fibrosis stage $\geq$ 2 grouped by metabolic indicators (HbA1c or BMI) with consistent sensitivity or specificity was then conducted. When the sensitivity or specificity was consistent between subgroups, the cut-off value of significant fibrosis (stage $\geq$2) for patients with HbA1c $\geq$ 7% or BMI $\geq$ 30 kg/m$^2$ was higher than those with HbA1c <7% or BMI <30 kg/m$^2$, as shown in Table 4.

**Correlation analysis of LSM value with other histologic parameters**

Spearman correlation analysis was performed to investigate the relationship between the LSM value of FibroScan and other pathologic degrees, as shown in Additional file 1- Table 2. The LSM value was correlated with the grade of ballooning ($r = 0.258, P = 0.007$) and inflammation ($r = 0.241, P = 0.013$). In addition, the distribution of LSM values classified in accordance with other histological parameters is shown in Additional file 2- Fig. 2. There was a stepwise increase trend as liver ballooning was aggravated, indicating that the LSM value can be used to roughly assess the severity of ballooning, but there was no significant trend in the LSM value with the increase in inflammation or steatosis degree.

**Discussion**

This is the first study that evaluated the diagnostic accuracy of FibroScan and obtained the cut-off value for NAFLD patients accompanied by abnormal glucose metabolism. Furthermore, the influence of metabolic indicators on FibroScan measurements was investigated. This cross-sectional study demonstrated that LSM values obtained using FibroScan achieved good diagnostic performance of liver fibrosis, especially in the more severe histologic stage, as the AUROC with a diagnosis of advanced fibrosis (stage $\geq$3) reached more than 80% and even 90% for cirrhosis (stage $\geq$4). It is worth noting that the NPV of stage $\geq$ 3 was more than 90%, indicating that FibroScan has high efficacy for excluding
advanced fibrosis and cirrhosis. In addition, this study suggests that HbA1c was independently associated with the LSM value. The glucose metabolism state may affect LSM value measurement, and its value was elevated in patients with poor glycaemic control, which further emphasized the significance of this study.

Several previous studies have investigated the accuracy of FibroScan in patients with NAFLD, but this study targeted people with NAFLD accompanied by abnormal glucose metabolism [18, 22, 23]. The LSM cut-off values in the study were within the interval of most previous studies, but the value of 8.3 kPa for diagnosing advanced fibrosis was lower than the 9.9 kPa recommended by the 2018 NAFLD guidance, which may be due to the differences in ethnicity and metabolic state [16]. All subjects in this study were Chinese, had a lower BMI and thinner subcutaneous fat thickness than Americans, and all had abnormal glucose metabolism. Consistent with previous studies, the results further confirmed that FibroScan is more suitable for assessing severe fibrosis, especially for ruling out advanced fibrosis, which can reduce the demand for liver biopsy to some extent.

It has been reported that several factors may affect FibroScan measurements, such as ALT, increased bilirubin levels, excessive alcohol intake and eating. Different cut-off values were suggested in patients with chronic liver disease caused by different aetiologies [24]. However, the effect of metabolic indicators on FibroScan has not been noticed thus far. Based on previous studies, there has been a hypothesis that metabolic indicators may affect the LSM value. As expected, after adjusting the liver pathological degree, HbA1c was significantly associated with the LSM value by multivariate linear regression analysis. There was a significant trend that the LSM value was higher in patients with HbA1c ≥7% than in those with HbA1c <7%. Although several studies have mentioned the effect of inflammation on FibroScan, and poorly controlled glucose metabolism may lead to more severe liver inflammatory activity, the trend and statistical significance still existed after matching the pathological inflammation and ballooning to counteract their influences. These results implied that inflammation was not the main factor contributing to the effect of glucose metabolism on LSM in this study. However, the potential mechanisms involved remain unknown and need to be further explored. In addition, the LSM cut-off value for significant fibrosis among patients with HbA1c ≥7% was higher than those with HbA1c <7% in the case of consistent specificity, which meant that different cut-off values should be adopted among patients with different glucose metabolism states. In addition, the AST level, a typical marker of liver inflammatory activity, was also an independent factor associated with the LSM value in multivariate linear regression analysis in this study. Factors such as hepatocyte swelling, inflammation, and oedema may increase the hydrostatic pressure of the liver in a short time, which results in an increased LSM value [31]. BMI and obesity were reported as independent risk factors for unreliable measurements as well as measuring failure [32]. A prospective multicentre study pointed out that a skin capsular distance (SCD) ≥25 mm led to overestimation of the LSM value [33]. In this study, BMI also affected the LSM measurement, no statistical significance may be due to the small sample size. The cut-off value among patients with BMI ≥30 kg/m² was higher than that among patients with BMI <30 kg/m² when the sensitivity between these two subgroups were consistent. The effect of BMI may be attributed to its influence on both
subcutaneous fat thickness and metabolic state. In general, the study provided a new perspective that glucose metabolism may influence the LSM value obtained from FibroScan. The current optimal cut-off values may be unsuitable for patients with abnormal glucose metabolism. Different cut-off values need to be considered for different glucose metabolic states in clinical applications. This is also the reason specific cut-off values are given for this study population. Clinically, stratified assessment of LSM based on glucose metabolic state is more practical. However, the results need to be further confirmed by expanding the subject number in the future.

**Study strengths and limitations**

The strengths of this study were that the degree of fibrosis was obtained from liver biopsy, a gold standard, and it is the first study to propose the effect of glucose metabolism on the LSM value and prove it to a certain extent. However, there were several limitations in the study. First, the data featured skewed distribution. The majority of patients involved have moderate NASH. There was a relative lack of data for patients with NAFL and severe fibrosis, as liver biopsy is not recommended by the guidelines for these parts of the population. The other limitation was that subgroup analysis revealed a general trend, but due to the small sample size, statistical significance was not detected in some subgroup comparisons. A similar analysis needs to be conducted by expanding the number of subjects in the future. In addition, more detailed subgrouping and cut-off value comparisons for each histologic degree can be carried out.

**Conclusions**

In conclusion, FibroScan was confirmed to be a relatively accurate diagnostic approach for evaluating liver fibrosis among NAFLD patients with abnormal glucose metabolism. It is valuable for evaluating severe fibrosis, especially for excluding advanced fibrosis. The glucose metabolic state may affect the LSM value, and the LSM values are higher in patients with poor glycaemic control. This study provides clinical advice for the application of FibroScan in the diagnosis and evaluation of NAFLD patients, especially in patients with abnormal glucose metabolism. It is worth noting that the glucose metabolism state should be considered in the clinical application of FibroScan.

**Abbreviations**

NAFLD, nonalcoholic fatty liver disease; HS, hepatic steatosis; NAFL, nonalcoholic fatty liver; NASH, nonalcoholic steatohepatitis; DM, diabetes mellitus; T2DM, type 2 diabetes mellitus; LSM, liver stiffness measurement; ALT, alanine aminotransferase; NAS, The NAFLD activity score; IQR, interquartile range; ROC curve, receiver operating characteristic curve; AUROC, area under the ROC curve; PPV, positive predictive value; NPV, negative predictive value; BMI, body mass index; HbA1c, haemoglobin A1c; TG, triglyceride; TC, total cholesterol; AST, aspartate aminotransferase

**Declarations**
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Author contributions

Authors’ contributions are as follows – XY Yang: research design, statistical analyses and interpretation of the data, drafting and revision of the manuscript; XX. Chang and SD. Wu: collection of the data, technical support; XY. Sun, XP. Zhu, L. W and YS. Xu: collection of the data and assistance in data analysis; XZ. Yao and SX. Rao: imaging technical support; XQ. H: pathological assessment; MF. Xia, H. Bian and HM. Yan: research design and conduction, interpretation of the data, critical revision of the manuscript; X. Gao: general director of research, research design and conduction, study supervision. The final manuscript was reviewed and approved to submit by all authors.

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Availability of data and materials

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Because only the medical records were reviewed, this case series was exempted from signing informed consent, which was approved by the Human Research Ethics Committee of Zhongshan Hospital Clinic (B2013-132).

Consent for publication

Not applicable.

Competing interests
The authors declare no conflicts of interest for the research conducted in this study.

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Tables

Table 1. Basic characteristics and hepatic histopathology of the patients
| Characteristics       | NAFLD                      |
|-----------------------|----------------------------|
| Total                 | 91                         |
| Sex, Male/Female      | 46/45                      |
| Age, y                | 40 (32-56)                 |
| Weight, kg            | 81.55 ± 15.32              |
| BMI, kg/m²            | 29.10 ± 4.06               |
| Waist–hip ratio, %    | 0.94 ± 0.06                |
| Hypertension, %       | 29 (31.9%)                 |
| Platelet, /10^5 µL    | 238.34 ± 70.05             |
| Fasting glucose, mmol/L | 5.8 (5.1-7.0)          |
| 2 h glucose, mmol/L   | 12.47 ± 3.82               |
| Haemoglobin A1c, %    | 6.7 (5.8-8.1)              |
| Triglycerides, mmol/L | 1.84 (1.23-2.68)           |
| Total cholesterol, mmol/L | 4.39 (3.89-5.14)   |
| LDL cholesterol, mmol/L | 2.57 ± 0.85              |
| HDL cholesterol, mmol/L | 0.99 (0.86-1.12)          |
| Albumin, g/L          | 44 (42-47)                 |
| Alanine aminotransferase, U/L | 61 (43-89)         |
| Aspartate aminotransferase, U/L | 37 (27-49)          |
| C-reactive protein, mg/L | 1.9 (1.1-3.7)           |
| LSM, kPa               | 8.5 ± 3.5                  |
| Steatosis grade, n (%)|                           |
| 1 5%-33%              | 18 (20.2)                  |
| 2 33%-66%             | 52 (58.4)                  |
| 3 >66%                | 19 (21.4)                  |
| Lobular inflammation, n (%) |                     |
| 0 None                | 3 (3.4)                    |
| 1 <2 foci per 200× field | 35 (39.3)              |
| 2 2-4 foci per 200× field | 38 (42.7)               |
| 3 >4 foci per 200× field | 13 (14.6)               |
| Liver cell ballooning, n (%) |                   |
| 0 None                | 2 (2.3)                    |
| 1 Few balloon cells   | 14 (15.7)                  |
| 2 Many balloon cells  | 73 (82.0)                  |
| NAFL/NASH, n          | 13/76                      |
| Fibrosis stage, n (%) |                           |
| 0 None                | 8 (8.8)                    |
| 1 Perisinusoidal or periportal | 33 (36.2)         |
| 2 Perisinusoidal and portal/periportal | 30 (33.0)      |
| 3 Bridging fibrosis   | 15 (16.5)                  |
| 4 Cirrhosis           | 5 (2 diagnosed with ultrasound) (5.5) |

All data are expressed as the mean ± SD, medians (interquartile range), or n (%), as appropriate.

*Abbreviation: BMI, body mass index; LSM, liver stiffness measurement.

### Table 2. Diagnostic accuracy of the LSM value in detecting each degree of fibrosis

| Fibrosis Stage | Cut-off value† (kPa) | AUROC | 95% CI | Se (%) | Sp (%) | PPV (%) | NPV (%) | P value |
|----------------|----------------------|-------|--------|--------|--------|---------|---------|---------|
| ≥1             | 6.3                  | 0.793 | 0.695-0.871 | 71.1   | 75.0   | 96.7    | 20.0    | <0.0001 |
| ≥2             | 7.6                  | 0.764 | 0.663-0.846 | 68.0   | 68.3   | 72.3    | 63.6    | <0.0001 |
| ≥3             | 8.3                  | 0.837 | 0.744-0.906 | 80.0   | 76.1   | 48.5    | 93.1    | <0.0001 |
| ≥4             | 13.8                 | 0.902 | 0.822-0.955 | 80.0   | 94.2   | 44.4    | 98.8    | <0.0001 |

†Cut-off value was optimized by the maximal sum of sensitivity and specificity.
* Abbreviation: AUROC, area under the receiver operator characteristic curve; 95% CI, 95% confidence interval; Se, sensitivity; Sp, specificity; PPV, positive predictive value; NPV, negative predictive value.

Table 3. Multivariate linear regression analysis of metabolic indicators associated with LSM value

| Variable | Standardized coefficients (β) | P value |
|----------|-------------------------------|---------|
| Fibrosis | 0.460                         | <0.001  |
| HbA1c    | 0.200                         | 0.038   |
| AST      | 0.200                         | 0.044   |
| BMI      | 0.164                         | 0.092   |
| Constant |                               | 0.563   |

The LSM value served as the dependent variable, and age, BMI, hypertension, fasting glucose, 2 h glucose, HbA1c, TC, TG, LDL cholesterol, HDL cholesterol, AST and ALT served as the independent variables after adjustment for liver fibrosis, ballooning and inflammation.

* Abbreviations: HbA1c, haemoglobin A1c; AST, aspartate aminotransferase; BMI, body mass index.

HbA1c and AST were independently associated with the LSM value after adjustment for the liver pathological degree.

Table 4. LSM cut-off value in detecting liver fibrosis stage ≥2 grouped by metabolic indicators with consistent sensitivity/specificity

| Subgroup       | Cut-off value† (kPa) | Se (%) | Sp (%) | PPV (%) | NPV (%) |
|----------------|----------------------|--------|--------|---------|---------|
| BMI <30 kg/m²  | 6.2                  | 81.5   | 50.0   | 59.5    | 75.0    |
| BMI ≥30 kg/m²  | 7.5                  | 81.8   | 63.6   | 81.8    | 63.6    |
| HbA1c <7%      | 7.7                  | 61.5   | 80.00  | 76.2    | 66.7    |
| HbA1c ≥7%      | 8.3                  | 60.9   | 80.00  | 82.4    | 57.1    |

† Cut-off value was determined when the sensitivity or specificity were consistent between subgroups.

* Abbreviation: 95% CI, 95% confidence interval; Se, sensitivity; Sp, specificity; PPV, positive predictive value; NPV, negative predictive value.