Performance of Liver Stiffness Measurement from FibroScan was 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

Research
Abstract

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

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

**Results:** The areas under ROC curves (AUROCs) of LSM values in diagnosing 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 HbA1c (β=0.200, p=0.038) and AST (β=0.200, p=0.044) were independently associated with LSM value after adjustment for fibrosis stage from liver biopsy. Subgroup analysis revealed that LSM values were slightly higher in patients with HbA1c ≥ 7% than those HbA1c < 7%, and in patients with BMI ≥ 30 kg/m² than those BMI < 30 kg/m².

**Conclusions:** FibroScan was valuable for the evaluation of liver fibrosis in NAFLD patients with abnormal glucose metabolism. It was recommended for evaluating severe fibrosis, especially for excluding advanced fibrosis. Glucose metabolism state may affect LSM value.

Introduction

Nonalcoholic fatty liver disease (NAFLD) has become the predominant cause of chronic liver injury worldwide, which refers to the presence of ≥ 5% hepatic steatosis (HS) without other competing etiologies, including chronic viral hepatitis, excessive alcohol consumption, use of steatogenic medication or hereditary disorders [1]. Its spectrum ranges from nonalcoholic fatty liver (NAFL), nonalcoholic steatohepatitis (NASH) to cirrhosis or even carcinoma, and is associated with the features of metabolic syndrome such as hypertension, insulin resistance, diabetes mellitus (DM) and dyslipidemia, increases risks of cardiovascular disease, and accelerates the progression of underlying disease, leading to severe consequences [2–4]. NALFD patients with DM is 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 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 our recent study, fibrosis occurred in up to 50% of patients with both NAFLD and T2DM [10]. Considering the huge number of patients with T2DM,
the burden of the management of NAFLD seems to be enormous. Although the effective therapies for NAFLD has not been established, early intervention can significantly improve the poor prognosis of NAFLD. Therefore, the accurate and early detection of NAFLD in patients with abnormal glucose metabolism, especially the staging of liver fibrosis, is crucial [8, 11, 12].

Liver biopsy, an invasive procedure, has been recommended as the gold standard for 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, non-invasive alternatives to liver biopsy have been investigated, such as serum biomarkers, clinical scoring systems and imaging tests including ultrasonography, FibroScan, magnetic resonance elastography (MRE), proton magnetic resonance spectroscopy ($^1$H-MRS) and magnetic resonance imaging-derived proton density fat fraction (MRI-PDFF) etc. [16, 17] Among them, FibroScan (EchoSens, Paris, France) is recommended by the 2018 NAFLD guidance because of its convenience, clinically 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].

There exist several studies assessing diagnostic performance of FibroScan, most of which targeted at 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 etiologies, consists of Hepatitis B, Hepatitis C and NAFLD etc. Factors such as liver inflammation activity manifested by alanine aminotransferase (ALT) or increased bilirubin levels, excessive alcohol intake and eating may lead to increase of LSM values [24]. In view of the promoting effect of abnormal glucose metabolism on liver disease, NAFLD patients with abnormal glucose metabolism may have specific cut-off values differentiated from general NAFLD patients. However, up to now, there are no studies focusing on these population and lack of cut-off values for them.

The aim of this study was to evaluate the diagnostic performance of FibroScan and obtain cut-off values in NAFLD patients with abnormal glucose metabolism, and investigate whether metabolic indicators would affect the measurement of FibroScan, in order to provide a 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 were identified cirrhosis using 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 study was approved by the Human Research Ethics Committee of Zhongshan Hospital Clinic and the whole process was conducted to the ethical guidelines of the Declaration of Helsinki. Written informed consent was also obtained from all patients.

**Liver biopsy and histopathologic evaluations**

Liver biopsy samples were obtained from right liver lobe of NAFLD patients using 16-gauge needle under ultrasound guidance. 2 specimens were obtained from each person to ensure sufficient sample size 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 according to Non-Alcoholic Steatohepatitis Clinical Research Network Scoring System [25]. Liver steatosis was categorized as grade 1, grade 2 and grade 3, while fibrosis was staged from stage 0 to stage 4 in accordance with the scoring system above. The NAFLD activity score (NAS) is the sum of scores for hepatic steatosis (grade 0–3), hepatocyte ballooning (grade 0–2) and lobular inflammation (grade 0–3). Another SAF scoring system was also adopted, consists of steatosis (grade 0–3), activity (grade 0–4) and fibrosis (stage 0–4). The classification criteria are described in the Table 1.
| 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, /10⁵ uL               | 238.34 ± 70.05         |
| Fasting glucose, mmol/L         | 5.8 (5.1–7.0)          |
| 2 h glucose, mmol/L             | 12.47 ± 3.82           |
| Hemoglobin A1c, %               | 6.7 (5.8–8.1)          |
| Total cholesterol, mmol/L       | 4.39 (3.89–5.14)       |
| Triglycerides, mmol/L           | 1.84 (1.23–2.68)       |
| 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 (%)     |                       |

All data are expressed as mean ± SD, medians (interquartile range), or n (%), as appropriate. BMI, body mass index; LSM, liver stiffness measurement.
| Characteristics                          | NAFLD   |
|-----------------------------------------|---------|
| 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 mean ± SD, medians (interquartile range), or n (%), as appropriate. BMI, body mass index; LSM, liver stiffness measurement.

**LSM measurement**

LSM measurement was performed with the FibroScan using M probe. Details of measurement are described in several previous studies, performed with the same machine by the same experienced operator blinded to other non-invasive methods and biopsy results [23, 26]. The whole examination duration was less than 5 minutes. 10 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 as reliable only when the success rate was ≥ 60% and the interquartile range (IQR)/median was ≤ 30%. Median value was kept as representative result. Liver stiffness measurement results are expressed with kilopascal (kPa).

**Basic characteristics collection**

Medical history of each patient was collected when admitted, including general physical characteristics, history of chronic diseases etc. Hypertension was diagnosed according to criteria [27]. Fasting blood samples were collected locally and then shipped to clinical laboratory of Zhongshan Hospital for assessment of the blood glucose, lipid profiles and other blood biochemical parameters.
Statistical analysis

Continuous variables with normal distribution were summarized as mean ± SD while those without were described with median (interquartile range). Categorical variables were summarized as frequencies and percentages. The software SPSS (version 23.0) was used for data analysis. The graphs were performed with Graphpad (version 8.4) and Medcalc (version 19.1). The receiver operator characteristic (ROC) curves were performed. The diagnostic accuracy was evaluated by the areas under ROC curves (AUROCs) and the optimal cut-off values were defined by Youden's index, with sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) calculated. The unpaired t test and Kruskal-Wallis test with Dunn's multiple correction were used for univariate comparisons between groups. The independent correlation was analyzed using multivariate linear regression analysis. The Spearman's rank correlation coefficient was used to assess the correlation between the liver histopathologic degree and FibroScan results in patients underwent biopsy. 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 were 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 stage 0, 1, 2, 3, 4 were 6.15, 6.60, 7.70, 10.50 and 14.60 kPa, respectively. The results are shown in Fig. 1A and it 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 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 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), 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 of liver fibrosis in NAFLD patients with abnormal glucose metabolism. The results were 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 FibroScan is an ideal machine to evaluate the severity of liver fibrosis, especially in more severe degrees. Besides, the cut-off value for each stage with sensitivity ≥ 90% or specificity ≥ 90% were added in Additional file 1- Table 1.
Table 2
Diagnostic accuracy of 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    | 0.0002  |
| ≥ 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|

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.

†Cut-off value was optimized by the maximal sum of sensitivity and specificity.

Influence of metabolic indicators on FibroScan in detecting liver fibrosis

We next investigated whether metabolic state would affect FibroScan measurements. The relationship between metabolic indicators including age, BMI, hypertension, fasting glucose, 2 h glucose, hemoglobin A1c (HbA1c), total cholesterol (TC), triglycerides (TG), LDL cholesterol, HDL cholesterol, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and LSM value was investigated using multivariate linear regression analysis after adjustment of liver fibrosis, as shown in Table 3. The results demonstrated that HbA1c (β = 0.200, p = 0.038) and AST (β = 0.200, p = 0.044) were independently correlated with LSM value after adjustment for the liver fibrosis stage. BMI (β = 0.164, p = 0.092) also entered the regression model, while no statistical significance was obtained. In order to further investigate the influence of metabolic indicators on FibroScan measurements, the subgroup analysis of LSM value was conducted grouped by BMI < 30 kg/m² or BMI ≥ 30 kg/m² and HbA1c < 7% or HbA1c ≥ 7%. The fibrosis stage was divided into stage 0–1 and stage 2–4 (significant fibrosis). The violin plots in Fig. 2A-B demonstrated a trend that LSM values were slightly higher for patients with BMI ≥ 30 kg/m² than those for patients with BMI < 30 kg/m², and statistically significant was detected in patients with significant fibrosis. Similar increase trend was also observed in patients with HbA1c ≥ 7% than compared with those HbA1c < 7%, and statistically significant was detected in patients without significant fibrosis. In view of the results above, we then conducted the subgroup comparison of cut-off value. When the sensitivity or specificity were consistent between subgroups, the cut-off value of patients with BMI ≥ 30 kg/m² or HbA1c ≥ 7% was higher than those without them, as shown in Table 4.
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                         | 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 variable after adjustment of liver fibrosis.

HbA1c, Hemoglobin A1c; AST, aspartate aminotransferase; BMI, body mass index.

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

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    |

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

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

Correlation analysis of LSM value with other histologic parameters

We also investigated the relationship between LSM value of FibroScan and other pathologic degrees, which was performed by Spearman correlation analysis, as shown in Additional file 1- Table 2. 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 value classified in accordance with other histological parameters was performed in Additional file 2- Fig. 1. There was a trend of stepwise increase as the liver ballooning aggravated, indicated that LSM value can be used to roughly assess the severity of ballooning, but no significant trend in LSM value with the increase of inflammation or steatosis degree.
Discussion

To the best of our knowledge, 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, we investigated the influence of metabolic indicators on FibroScan measurement. 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 ≥ 3) reached up to more than 80% and even 90% for cirrhosis (stage ≥ 4). It is worth noting that the NPV of stage ≥ 3 was more than 90%, indicated that FibroScan has high efficacy for excluding advanced fibrosis and cirrhosis. In addition, our study suggested that HbA1c was independently associated with LSM value. Glucose metabolism state may affect LSM value measurement, and its value was elevated in patients with poor glycemic control, which further emphasized the significance of this study.

Several studies have previously investigated the accuracy of FibroScan in patients with NAFLD, but our study targeted at people with NAFLD accompanied by abnormal glucose metabolism [18, 22, 23]. The LSM cut-off values in our study were within the interval of most previous studies, but the value 8.3 kPa for diagnosing advanced fibrosis was lower than 9.9 kPa recommended by the 2018 NAFLD guidance, which may be due to the differences of ethnicity and metabolic state [16]. All subjects in this study are Chinese, with lower BMI and thinner subcutaneous fat thickness than American, and all of them were accompanied with abnormal glucose metabolism. Consistent with previous studies, our 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 the FibroScan measurement, 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 etiologies [24]. However, the effect of metabolic indicators on FibroScan has not been noticed so far. Based on previous studies, we hypothesized that metabolic indicators may affect LSM value. As we expected, after adjusting liver fibrosis stage, the HbA1c and AST were independently associated with LSM value by multivariate linear regression analysis. The effect of glucose metabolism on LSM measurement may be due to the fact that T2DM patients have more severe liver inflammation, and LSM value is closely associated with inflammatory injury [28]. Similarly, AST level was a typical marker of liver inflammation activity. BMI and obesity were reported as independent risk factors of unreliable measurements as well as measuring failure [29]. A prospective multicenter study pointed out that skin capsular distance ≥ 25 mm lead to overestimation of LSM value [30]. In our study, BMI also affect the LSM measurement but no statistical significance was obtained because of the small sample size. Its effect may be attributed to influence on both subcutaneous fat thickness and metabolic state. In addition, there was a trend that the LSM values of patients with BMI ≥ 30 kg/m² or HbA1c ≥ 7% was slightly higher than patients without them. The cut-off values of these two subgroups also revealed increase trends in the case of consistent sensitivity or specificity. As the M probe is still the main tool for LSM measurement, the current optimal cut-off values are unsuitable for patients with abnormal glucose
metabolism. Different cut-off values need to be considered for different BMI or different metabolic state in clinical application. This is also the reason why we gave specific cut-off values for this population in our study. The results need to be further confirmed by expanding subject number in the future.

Our study had several limitations. Firstly, 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 guideline for these parts of the population. The other limitation was that subgroup analysis revealed a general trend, but due to the small sample size, statistically significance was not detected in some subgroup comparisons. Similar analysis needs to be conducted by expanding the number of subjects in the future. Besides, more detailed subgrouping and the cut-off value comparison for each histologic degree can be carried out.

In conclusion, FibroScan is 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. Glucose metabolism state should be considered in clinical application of LSM measurement.

**Abbreviations**

LSM, liver stiffness measurement; NAFLD, nonalcoholic fatty liver disease; ROC curves and AUROCs, receiver operating characteristics curves and areas under the curves; HS, hepatic steatosis; NAFL, nonalcoholic fatty liver; NASH, nonalcoholic steatohepatitis; DM and T2DM, diabetes mellitus and type 2 diabetes mellitus; IQR, interquartile range; BMI, body mass index; HbA1c, hemoglobin A1c; TC, total cholesterol; TG, triglycerides; ALT, alanine aminotransferase; AST, aspartate aminotransferase

**Declarations**

**Acknowledgments**

We gratefully acknowledge Huandong Lin (Department of Endocrinology and Metabolism, Zhongshan Hospital, Fudan University) for funding support of Xiamen Bureau of Science and Technology [3502Z20209043 to Lin HD], Lili Liu, Qunyan Yao, Ningping Zhang (Department of Gastroenterology, Zhongshan Hospital, Fudan University) for skillful technical assistance.

**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. All authors read and approved the final manuscript.

**Funding**

This work was financially supported by the Shanghai Municipal Population and Family Planning Commission [201740092 to HM Yan]; the Special Project of Integrating Traditional Chinese and Western Medicine in Shanghai General Hospital from the Shanghai Municipal Population and Family Planning Commission and Shanghai TCM Development Office [ZY (2018-2020)-FWTX-3019 to HM Yan]; the Foundation of Fudan University, China (20520133483 to HM Yan); Science and Technology Commission of Shanghai Municipality [20ZR1410200 to H Bian] Xiamen Municipal Bureau of Science and Technology [3502Z20209043 to HD Lin].

**Availability of data and materials**

The datasets generated during and/or analyzed 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 the informed consent, which had been approved by the Human Research Ethics Committee of Zhongshan Hospital Clinic (B2013-132).

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare no conflict of interest for the research conducted in this study.

**References**

1. Friedman SL, Neuschwander-Tetri BA, Rinella M, Sanyal AJ. Mechanisms of NAFLD development and therapeutic strategies. Nat Med. 2018;24:908-22.
2. Anstee QM, Mantovani A, Tilg H, Targher G. Risk of cardiomyopathy and cardiac arrhythmias in patients with nonalcoholic fatty liver disease. Nat Rev Gastroenterol Hepatol. 2018;15:425-39.

3. Adams LA, Anstee QM, Tilg H, Targher G. Non-alcoholic fatty liver disease and its relationship with cardiovascular disease and other extrahepatic diseases. Gut. 2017;66:1138-53.

4. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology. 2016;64:73-84.

5. Tilg H, Moschen AR, Roden M. NAFLD and diabetes mellitus. Nat Rev Gastroenterol Hepatol. 2017;14:32-42.

6. Williams CD, Stengel J, Asike MI, et al. Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: a prospective study. Gastroenterology. 2011;140:124-31.

7. Younossi ZM, Golabi P, de Avila L, et al. The global epidemiology of NAFLD and NASH in patients with type 2 diabetes: A systematic review and meta-analysis. J Hepatol. 2019;71:793-801.

8. Angulo P, Kleiner DE, Dam-Larsen S, et al. Liver Fibrosis, but No Other Histologic Features, Is Associated With Long-term Outcomes of Patients With Nonalcoholic Fatty Liver Disease. Gastroenterology. 2015;149:389-97 e310.

9. Hagstrom H, Nasr P, Ekstedt M, et al. Fibrosis stage but not NASH predicts mortality and time to development of severe liver disease in biopsy-proven NAFLD. J Hepatol. 2017;67:1265-73.

10. Bian H, Zhu X, Xia M, et al. Impact of Type 2 Diabetes on Nonalcoholic Steatohepatitis and Advanced Fibrosis in Patients with Nonalcoholic Fatty Liver Disease. Endocr Pract. 2020;26:444-53.

11. Bhati C, Idowu MO, Sanyal AJ, et al. Long-term Outcomes in Patients Undergoing Liver Transplantation for Nonalcoholic Steatohepatitis-Related Cirrhosis. Transplantation. 2017;101:1867-74.

12. Dulai PS, Singh S, Patel J, et al. Increased risk of mortality by fibrosis stage in nonalcoholic fatty liver disease: Systematic review and meta-analysis. Hepatology. 2017;65:1557-65.

13. Bedossa P, Dargere D, Paradis V. Sampling variability of liver fibrosis in chronic hepatitis C. Hepatology. 2003;38:1449-57.

14. Gaidos JKJ, Hillner BE, Sanyal AJ. A decision analysis study of the value of a liver biopsy in nonalcoholic steatohepatitis. Liver Int. 2008;28:650-8.

15. Bravo AA, Sheth Sg Fau - Chopra S, Chopra S. Liver biopsy. N Engl J Med. 2001;344:495-500.

16. Chalasani N, Younossi Z, Lavine JE, et al. The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. Hepatology. 2018;67:328-57.

17. Baumeler Stephana, Jochum Wolframb, Neuweiler Jörgb, Bergamin Irinama, Davida S. Controlled attenuation parameter for the assessment of liver steatosis in comparison with liver histology: a single-centre real life experience. Swiss Medical Weekly. 2019;149:w20077.
18. Imajo K, Kessoku T, Honda Y, et al. Magnetic Resonance Imaging More Accurately Classifies Steatosis and Fibrosis in Patients With Nonalcoholic Fatty Liver Disease Than Transient Elastography. Gastroenterology. 2016;150:626-37 e627.

19. Mikolasevic I, Orlic L, Franjic N, Hauser G, Stimac D, Milic S. Transient elastography (FibroScan(R)) with controlled attenuation parameter in the assessment of liver steatosis and fibrosis in patients with nonalcoholic fatty liver disease - Where do we stand? World J Gastroenterol. 2016;22:7236-51.

20. Shen F, Zheng RD, Mi YQ, et al. Controlled attenuation parameter for non-invasive assessment of hepatic steatosis in Chinese patients. World J Gastroenterol. 2014;20:4702-11.

21. Jung KS, Kim SU, Ahn SH, et al. Risk assessment of hepatitis B virus-related hepatocellular carcinoma development using liver stiffness measurement (FibroScan). Hepatology. 2011;53:885-94.

22. Tapper EB, Challies T, Nasser I, Afdhal NH, Lai M. The Performance of Vibration Controlled Transient Elastography in a US Cohort of Patients With Nonalcoholic Fatty Liver Disease. Am J Gastroenterol. 2016;111:677-84.

23. Eddowes PJ, Sasso M, Allison M, et al. Accuracy of FibroScan Controlled Attenuation Parameter and Liver Stiffness Measurement in Assessing Steatosis and Fibrosis in Patients With Nonalcoholic Fatty Liver Disease. Gastroenterology. 2019;156:1717-30.

24. Control; CFFHPa, Chinese Society of Infectious Disease and Chinese Society of Hepatology CMA, Association LDCoCRH. Consensus on clinical application of transient elastography detecting liver fibrosis: a 2018 update. Chinese Journal of Hepatology. 2019;27:182-91.

25. Kleiner DE, Brunt EM, Van Natta M, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology. 2005;41:1313-21.

26. Sandrin L, Fourquet B, Hasquenoph JM, Yon S, Fournier C, Mal F, et al. Transient elastography: a new noninvasive method for assessment of hepatic fibrosis. Ultrasound in Med. & Biol. 2003;29:1705-13.

27. Unger T, Borghi C, Charchar F, et al. 2020 International Society of Hypertension global hypertension practice guidelines. J Hypertens. 2020;38:982-1004.

28. Zeng X, Xu C, He D, et al. Influence of Hepatic Inflammation on FibroScan Findings in Diagnosing Fibrosis in Patients with Chronic Hepatitis B. Ultrasound Med Biol. 2015;41:1538-44.

29. Wong GL, Wong VW, Chim AM, et al. Factors associated with unreliable liver stiffness measurement and its failure with transient elastography in the Chinese population. J Gastroenterol Hepatol. 2011;26:300-5.

30. Shen F, Zheng RD, Shi JP, et al. Impact of skin capsular distance on the performance of controlled attenuation parameter in patients with chronic liver disease. Liver Int. 2015;35:2392-400.

**Supplementary Files**

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfile1.docx