Evaluation and Diagnosis of Nonalcoholic Fatty Liver Disease/Steatohepatitis by K-Fold Validation Based on Pathology, Including Real-Time Shear Wave Elastography and Noninvasive Biomarkers

Jie Zhou
West China Hospital of Sichuan University
Feng Yan
Laboratory of Ultrasound Imaging of West China Hospital of Sichuan University
Jinshun Xu
Laboratory of Ultrasound Imaging of West China Hospital of Sichuan University
Qiang Lu
West China Hospital of Sichuan University
Xianglan Zhu
West China Hospital of Sichuan University
Binyang Gao
Laboratory of Ultrasound Imaging of West China Hospital of Sichuan University
Huan Zhang
West China Hospital of Sichuan University
Rui Yang
Laboratory of Ultrasound Imaging of West China Hospital of Sichuan University
Yan Luo (luoyanddoc@163.com)
West China Hospital of Sichuan University

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Abstract

Objectives

This study aim to investigate the diagnostic accuracy of shear wave elastography (SWE) for diagnosing nonalcoholic steatohepatitis (NASH) and staging fibrosis in a cohort patients confirmed nonalcoholic fatty liver disease (NAFLD) by liver biopsy.

Methods

A total of 86 NAFLD patients and 17 normal-control were enrolled. The performance of SWE to diagnose NASH and stage fibrosis was evaluated on the basis of histopathological inflammation grades and fibrosis stages according to Kleiner/Brunt et al.'s criteria classification, and compared to previous reported four noninvasive serum fibrotic scores, coupled with the k-fold-cross-validation and Delong test. Meanwhile, influence of steatosis on liver stiffness measurements (LSMs) of SWE was also studied.

Results

LSMs of SWE proved to be an excellent diagnostic indicator for detecting NASH (AUROC□0.85), and fibrotic NASH: ≥F2 stage (AUROC□0.92), ≥F3 stage (AUROC□0.94) and =F4 stage (AUROC □0.94) with the cutoff values were 7.55, 7.65, 8.25 and 11.80 kPa, respectively. Compared with serum fibrotic scores, SWE had the highest AUROC for predicting ≥F2, ≥F3, =F4 by Delong test (all P□0.05). No statistic differences of LSMs were found among different steatosis levels (P=0.29).

Conclusion

The stiffness reconstructions based on SWE could be used to noninvasively identify NASH and stage fibrosis in NAFLD patients. Moreover, the diagnosis efficiency of LSMs on SWE could not be influenced by steatosis.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD), defined by the presence of steatosis in > 5% of hepatocytes, is a spectrum of diseases strongly associated with insulin resistance [1]. In recent years, NAFLD prevalence is growing rapidly [2, 3] and has become most common cause of chronic liver diseases in the world [4, 5]. Based on histopathological features of hepatocellular damage, NAFLD encompasses a wide spectrum ranging from benign nonalcoholic fatty liver (NAFL), progressive non-alcoholic steatohepatitis (NASH), to cirrhosis finally [1]. Owing to NAFL usually remains stable for years without progression in most patients [6–8], but NASH always indicates an increased risk of fibrosis progression, cirrhosis and possibly hepatic comorbidities (such as hepatic failure, hepatocellular carcinoma) [9, 10]. It is important to accurately differentiate NASL and NASH, which is attributed to formulate the optimum options for monitoring and treatment in NAFLD patients [11].

Liver biopsy as the gold standard has long been considered for assessing the stage of NAFLD. However, it is invasive, difficult to accept, and inconvenient for the repeat measurement of treatment reaction [1]. Thereby, several non-invasive methods have been studied in the last decade, including cytokeratin-18 fragments (CK-18) [12], NAFLD fibrosis score (NFS) [13], fibrosis 4 calculator (FIB-4) [14], and liver stiffness measurements (LSMs) based on transient elastography (TE) imaging [15, 16], demonstrating good capabilities for diagnosis of NAFL, NASH, and advanced fibrosis. However, none of them can be used to substitute liver biopsy based on the convergent findings from recently critical appraisals [17, 18]. Furthermore, there has been no consensus on thresholds or strategies for use in non-invasive methods when trying to avoid biopsy according to the guidelines from European Association for the Study of the Liver (EASL) [1, 18]. Consequently, more preferable strategies are urgently needed to improve the capability of the non-invasive measurements for diagnosis of NAFLD subtypes.

Shear wave elastography (SWE) based on a radiation force induced by focused ultrasonic beams in tissues has many advantages, including realtime, convenience to implement, high range of values (2-150 kPa), and high performance for diagnosis, etc. [18]. Recently, use of SWE has been reported to be effective for accurate diagnosis of fibrosis in various liver

diseases [19–24]. Nevertheless, little research has been investigated in NASH patients. In addition, steatosis and fibrosis would co-exist in the same NAFLD patient. Thereby, the effect of steatosis on LSMs of SWE also needs to be assessed.

Hence, the purpose of this study is to (1) validate the diagnostic accuracy of SWE for NASH and quality criteria using histopathology as the reference; (2) evaluate and compared the accuracy of SWE and four previous reported noninvasive serum fibrosis scores (APRI, FIB-4, NFS and BARD scores) for further staging fibrosis in NASH patients; (3) investigate the influence of steatosis on LSMs of SWE in all NAFLD patients.

2. Materials And Methods

2.1. Patients

All the patients' private information is not included in the paper. We included patients aged 18 years or older in West China hospital from September 2015 to September 2019, whom pathologic examination confirmed the accumulation of lipid within more than 5% hepatocytes. All patients underwent liver biopsy and liver SWE measurement consecutively in one day. The exclusion criteria were showed in supplemental materials (1.Supplemental exclusion criteria of NAFLD and normal control group).

2.2. Clinical and Laboratory Assessments

Relevant clinical data: sex, age, weight, height, and alcohol intake (g/day), and laboratory results: total bile acid (TB), direct bile acid (DB), blood glucose, triglyceride (TG), alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), lactic dehydrogenase (LDH) were collected at the time of liver biopsy. Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared.

NFS was calculated according to the following formula: -1.675 0.037 × age (years) 0.094 × BMI (kg/m2) impaired fasting glycemia or diabetes (yes=1; no 0) 0.99 × AST/ALT 0.013 × platelet (109/L) 0.66 × albumin (g/dL) [13]. FIB-4 score was calculated as follows: age × AST (U/L)/platelet count (109/L)×√ALT (U/L) [14]. BARD score was the weighted by sum of three variables (BMI 28 1 point; AST/ALT ratio ≥ 0.8 2 points; and diabetes 1point) [25]. APRI scoring formulation was AST level (/upper limit of normal value[ULN]) /PLT (109/L) × 100 [26].

2.2. US examination and SWE

All subjects underwent ultrasound examination by SuperSonic Imagine AixPlorer (SuperSonic Imagine, Aix-en-Provence, France) before fast for at least 8 hours. SWE was conducted using a convex probe (SC6-1) by a proficient sonographer performing ultrasound examinations, SWE measurement and needle biopsy. The examinees were placed in the supine position with the right-arm maximal extension. Two-dimension ultrasonic examination was first performed before select SWE mode. The transducer was positioned in the right lobe of the liver through the intercostal space (segment V, VIII, or VII) with the transducer at 90° in relation to the liver capsule in an area free of artifacts and large vessels. The region of interest was placed a minimum of 1–2 cm and a maximum of 6 cm below the liver capsule. The analysis box was set to at least 10 mm. The patients transiently held their breath in a neutral position. Five successful measurements were repeated. The mean of the five times SWE measurements expressed in kilopascals (kPa) was used as the representative measurement.

2.3. Histopathological assessment

After US and SWE examination, liver needle biopsy was performed in the same area of SWE examination. Then, The liver biopsy specimens were stained with hematoxylin-eosin (HE), Foot and Masson stains. The diagnostic performance of SWE was evaluated on the basis of histopathological inflammation and fibrosis stage according to Kleiner/Brunt et al.'s criteria classification [27–29] (2.Supplemental histopathological assessment).

2.4. Statistical analysis

SPSS 20.0 (IBM SPSS Statistics 20.0) statistical software and R (A language and environment for statistical computing, Vienna, Austria, ISBN 3-900051-07-0, URL http://www.R-project.org) was used for analysis. All continuous variables were confirmed

normal distribution or analyzed after normal-scores transformation and were expressed as mean ± standard deviations (SD), otherwise, median and IQR (25th-75th percentile) were reported. Data were analyzed by using the Student t test, the chi-square test, Fisher's exact test and analysis of variance, as appropriate. The data was firstly used for predicting NASH based on binary logistic regression. The variables with a $P < 0.05$ in the logistic regression were included. Then the data obtained were divided into train sets and test sets, a ratio of 4:1 by 5-fold cross validation of R to calculate the cutoff value, sensitivity (Sen), specificity (Spe), positive predictive value (PPV), negative predictive value (NPV), and area under the receiver operating characteristic curves (AUROCs). AUROCs were compared using the DeLong test [30].

3. Results

3.1. Patient characteristics

The institutional electronic database was collected from September 2015 to September 2019. The inclusion and exclusion flow chart of the retrospective study was depicted in Fig. 1. In all, 86 NAFLD patients and 17 normal control patients who fulfilled the study criteria were enrolled. The total of 103 patients had an age range from 18 to 77 years, 42.7% were male. In NAFLD patients group, 23 (26.7%) patients have no fibrosis based on histopathological examination, 32 (37.2%) patients had F1, 14 (16.3%) patients had F2, 5 (5.8%) patients had F3 and 12 (13.9%) had F4. The characteristics and histological findings of the independent study groups are shown in Table 1. Figure 2 showed the example of SWE images and HE stain sections.

Table 1
Clinical Characteristics and Laboratory Data of Patients

Characterastics	Normal controls(n = 17)	NAFLD (n = 86)	All patients (n = 103)	*P* value
Age(years)	48.1 ± 12.1	46.5 ± 16.8	46.7 ± 16.0	0.7
Gender(male)(%)	6(35.3%)	38(44.2%)	44(42.7%)	0.5
BMI(kg/m^2)	22.5 ± 2.5	25.0 ± 3.5	24.6 ± 3.5	0.01
Overweight(BMI ≥ 25 kg/m^2)(%)	4(8.5%)	48(55.8%)	52(50.5%)	0.03
Obesity(BMI ≥ 30 kg/m^2)(%)	0(0%)	9(10.5%)	9(8.7%)	0.17
Diabetes(n)(%)	1(5.9%)	9(10.5%)	10(10.3%)	0.89
Hypertension(n)(%)	6(35.3%)	26(30.2%)	32(31.1%)	0.68
Metabolic syndrome(n)(%)	2(11.8%)	6(7.0%)	8(7.7%)	0.86
ALT(IU/L)	22.2 ± 8.1	140.9 ± 209.0	119.9 ± 174.8	0.001
AST(IU/L)	23.9 ± 8.7	119.3 ± 221.4	102.4 ± 203.9	0.001
Gamma glutamyl transpeptidase(IU/L)	60.7 ± 58.9	216.6 ± 500.8	189.0 ± 458.4	0.23
Alkaline phosphatase(IU/L)	84.5 ± 28.1	163.2 ± 190.0	149.6 ± 175.1	0.12
Lactic dehydrogenase(IU/L)	180.6 ± 53.8	244.9 ± 193.0	233.5 ± 178.0	0.18
Hydroxybutyrate dehydrogenase(IU/L)	143.7 ± 46.7	182.1 ± 138.5	175.3 ± 127.8	0.26
Triglyceride(mmol/L)	1.1 ± 0.4	1.6 ± 0.8	1.5 ± 0.8	0.016
Cholesterol(mmol/L)	4.4 ± 0.8	4.8 ± 1.3	4.8 ± 1.2	0.195
Total bilirubin(mg/dl)	11.2 ± 4.1	23.7 ± 33.2	21.5 ± 30.5	0.105
Albumin(g/L)	45.1 ± 2.8	46.1 ± 8.0	45.9 ± 7.4	0.62
Platelet count(× 10^9/L)	176.5 ± 52.7	169.3 ± 67.4	170.6 ± 64.8	0.77
High-density lipoprotein(mmol/L)	1.5 ± 0.5	1.8 ± 0.9	1.7 ± 0.8	0.184
Low-density lipoprotein(mmol/L)	2.5 ± 0.6	2.2 ± 1.1	2.3 ± 1.0	0.139
Uric acid(umol/L)	299.4 ± 64.6	343.6 ± 107.5	334.7 ± 102.3	0.106
NASH level(0–1/2–3/4)	17/0/0	55/19/12	72/19/12	
Fibrosis stage(0/1/2/3/4)	17/0/0/0/0	23/32/14/5/12	40/32/14/5/12	
SWE(kPa)	5.9 ± 1.1	9.2 ± 4.3	8.7 ± 4.1	0.001

Note: the table shows the mean ± SD for normally distributed continuous variables or variables that underwent non-normal data distribution transformation, number (%) for binary variables. Abbreviation: BMI, body mass index; AST, aspirate aminotransferase; ALT, alanine aminotransferase; NASH, nonalcoholic steatohepatitis; SWE, real-time shear wave elastography.

3.2. Predictors of NASH.

The result of binary regression was showed in Table 2, only SWE have statistic difference with OR = 3.91 among multiple variables (P = 0.01). The LSMs (± SD) of NASH group (10.2 ± 4.5 kPa) were higher than both normal control (6.1 ± 0.9 kPa) and

NAFL group (6.2 ± 0.7 kPa) (P < 0.001) (Fig. 3). However, there was no statistical difference between the normal control (6.1 ± 0.9 kPa) and NAFL group (6.2 ± 0.7 kPa) (P = 0.26).

Table 2
Variables Associated with Presence of NASH in the NAFLD patients and normal control group

Variables	OR	95% CI		*P* value
		low	high	
Age	1.08	0.93	1.26	0.3
Gender(Male)	0.99	0.03	31.65	1
BMI	1.24	0.46	3.39	0.67
Overweight	3.59	0.02	552.03	0.62
Obese	0.03	0	56.12	0.37
Diabetes	0.01	0	62.57	0.29
Hypertension	0.24	0.01	11.4	0.47
Metabolic syndrome	1.00	0.23	4.73	0.85
ALT(IU/L)	1.04	0.98	1.03	0.81
AST(IU/L)	1.04	0.97	1.11	0.31
Triglyceride(mmol/L)	0.04	0	2.05	0.11
Cholesterol(mmol/L)	1.27	0.90	1.81	0.18
High-density lipoprotein(mmol/L)	0.03	0	11.88	0.25
Low-density lipoprotein(mmol/L)	0.03	0	3.76	0.15
Alkaline phosphatase(IU/L)	1.05	0.97	1.12	0.23
Gamma glutamy transpeptidase(IU/L)	0.99	0.96	1.03	0.74
Lactic dehydrogenase(IU/L)	1.01	0.93	1.1	0.75
Hydroxybutyrate dehydrogenase(IU/L)	0.94	0.81	1.09	0.4
Platelet count($\times 10^9$)	1.01	0.98	1.04	0.72
Hemoglobin(g/L)	1.03	0.93	1.14	0.62
Uric acid(umol/L)	1.02	1	1.04	0.13
Albumin(g/L)	1.32	0.87	2	0.2
SWE(kPa)	3.91	1.36	11.24	0.01
AAR	2.95	0.02	430.46	0.67

Note: Abbreviation BMI, body mass index; AAR, the ratio of AST to ALT; AST, aspirate aminotransferase; ALT, alanine aminotransferase; SWE, real-time shear wave elastography.

For the diagnosis of NASH by SWE and calculated optimal cutoff value, the data obtained were randomly divided into train sets and testing sets (ratio of 4:1) by caret package, and then analyzed by pROC package of R. As demonstrated in Fig. 4 and Table 3, for differentiated NASH from NAFL by SWE, the mean AUROC (95% CI) for 5-fold cross validation train sets was 0.85 (0.75–0.92). The mean Sen, Spe, PPV, NPV, and AC were 64.7%, 96.7%, 97.1%, 61.7% and 76.5%, respectively, when the cutoff

was 7.55 kPa. In test sets, the Sen, Spe, NPV, PPV, AC were 78.0%, 100.0%, 100.0%, 72.08% and 86.4%, respectively. These results demonstrated that SWE had a significantly high Sen, Spe, NPV, PPV and AC in both train and test sets for diagnosing NASH in NAFLD patients.

Table 3
Accuracy of SWE for diagnosing fibrosis in NAFLD patients

Stage	AUROC(95%CI)	Criteria	Cut-off(kPa)	DA(%)	Sen(%)	Spe(%)	PPV(%)	NPV(%)	-LR	+LR	DOR
≥F2	0.92(0.86–0.98)	BCV	7.65	83.5	93.5	79.2	65.9	96.6	0.08	4.49	3.82
		95 Sen	7.1	74.7	95	65.9	54.5	96.8	0.08	2.79	9.83
		95 Spe	10.85	85.4	61.3	95	86.4	85.2	0.40	14.71	0.07
≥F3	0.94(0.89–0.99)	BCV	8.25	80.6	94.1	77.9	45.7	98.5	0.08	4.26	4.54
		95 Sen	7.7	73.8	95	67.6	36.7	98.6	0.07	2.93	9.10
		95 Spe	11.8	91.3	70.6	95	75.0	94.3	0.31	15.2	0.12
=F4	0.94(0.89-1.0)	BCV	11.8	92.2	83.3	93.4	62.5	97.7	0.18	12.64	0.35
		95 Sen	8.25	76.5	95	74.1	35.6	99.1	0.07	3.67	6.65
		95 Spe	12.8	91.3	66.7	95	61.5	95.6	0.35	12.13	0.12

Note: Abbreviation AUROC, area under the receiver-operating characteristics curve; 95%CI, 95% confidence interval; BCV, the best diagnostic value; DA, diagnostic accuracy; Sen, sensitivity; Spe, specificity; NPV, negative predictive value; PPV, positive predictive value; -LR, negative likelihood ratio;+LR, positive likelihood ratio; DOR, diagnostic odds ratio

3.3. Predictors of significant fibrosis by SWE

Next, diagnostic performance of SWE in separating patients' fibrosis stage was evaluated. The mean LSMs (± SD) of NAFLD patients from F0 to F4 were 6.1 ± 0.9, 7.8 ± 2.3, 10.3 ± 2.7, 12.2 ± 3.6 and 16.3 ± 5.5 kPa, respectively. The above data demonstrated a stepwise increase with increasing severity of hepatic fibrosis. The LSMs of non-advanced fibrosis (F0-2) and advanced types (F3-4) had statistic difference (P□0.01). However, except for F0 and F1, the LSMs between two adjacent fibrosis stages had no statistic difference (P□0.05 for all) (Fig. 5).

For diagnosing ≥ F2, the best LSMs cutoff was 7.65 kPa in train sets with the AUROC of 0.92 (95%CI 0.87–0.99). For diagnosing F3 or greater disease, the best LSMs cutoff was 8.25 kPa in train sets with the AUROC of 0.94 (95%CI 0.87–0.99). The optimal cutoff LSMs for diagnosing F4 was 11.8 kPa, the AUROC showed 0.95 (95%CI 0.88-1). The Sen, Spe, PPV, NPV, AC and AUROC in train and test sets were demonstrated in Table 3.

3.4. Liver stiffness assessment: comparison of SWE, NFS, FIB-4, BARD and APRI scores

Performance of the four noninvasive serum fibrosis scores: NFS, FIB-4, BARD and APRI scores were also assessed by 5-fold cross validation and compared with SWE. Significantly higher AUROCs were observed for SWE than for the four noninvasive serum fibrosis score (NFS, FIB-5, BARD and APRI scores) in the identification of each fibrosis stage (≥ F2: 0.93 vs (0.76, 0.78, 0.55, 0.75); ≥F3 0.94 vs (0.84, 0.83, 0.57, 0.74); =F4: 0.95 vs (0.82, 0.74, 0.57, 0.77); all $P < 0.05$) (Table 4). Nevertheless, the optimal applications for stage fibrosis using NFS, FIB-4, BARD and APRI scores were different: APRI is appropriate to ≥ F2, NFS,

FIB-4 and BARD score are more suitable for applying to ≥ F3 [13, 14, 25, 26]. In fact, best application of four serum fibrosis scores also showed lower AUROCs for their respective optimum when compared with SWE (showed Table 5). Consequently, the LSMs on SWE was significantly superior (all $P < 0.05$) for diagnosis of liver fibrosis stage (F2-4), compared to the NFS, FIB-4, BARD and APRI scores respectively (Fig. 6).

Table 4
AUROCs of the non-invasive fibrosis tests and SWE

Fibrosis test	AUROC(95%CI)		
	≥F2	≥F3	F4
The NAFLD fibrosis score	0.43(0.31–0.55)	0.45(0.30–0.60)	0.35(0.20–0.51)
FIB-4	0.47(0.35–0.58)	0.51(0.37–0.65)	0.45(0.29–0.61)
BARD score	0.40(0.28–0.51)	0.43(0.29–0.57)	0.40(0.24–0.56)
APRI score	0.74(0.65–0.84)	0.73(0.60–0.85)	0.78(0.64–0.90)
SWE	0.92(0.86–0.98)	0.94(0.89–0.99)	0.94(0.89-1.0)

Table 5
The optimal diagnostic performance of noninvasive blood test and SWE in diferentiating NASH from NAFL and staging fibrosis

Methods	Best application	DA(%)	Sen(%)	Spe(%)	PPV(%)	NPV(%)	-LR	+LR	DOR
NFS	≥F3	92.2	52.9	100	100	91.5	0.47	\	0
FIB-4	≥F3	83.5	70.6	86.0	50	94.9	0.34	5.06	0.39
BARD score	≥F3	40.8	64.7	36.0	16.7	83.8	0.98	1.01	3.25
APRI	≥F2	62.1	32.3	75.0	35.7	72.0	1.33	1.90	0.16
SWE	NASH	81.6	81.3	82.1	88.1	72.7	0.23	4.53	0.95
	≥F2	83.5	93.5	79.2	65.9	96.6	0.08	4.49	3.81
	≥F3	80.6	94.1	77.9	45.7	98.5	0.08	4.26	4.53
	F4	92.2	83.3	93.4	62.5	97.7	0.18	12.64	0.35

3.5. The influence of steatosis and inflammation on LSMs

In this study, steatosis, lobular inflammation and fibrosis would coexist in NASH patient. To assess the effect of steatosis on LSMs, we stratified NAFL patients with different steatosis levels and NASH patients at each fibrosis stages with different inflammation grades. The LSMs (± SD) of NAFL patients with 5–33%, 33–66% and ≥ 66% of steatosis hepatocytes were 6.2 ± 0.6, 6.9 ± 0.5 and 6.0 ± 0.9 kPa, respectively, showing no statistic differences among different steatosis levels ($F = 1.3$, $P = 0.29$).

4. Discussion

The prognosis of NAFLD depends heavily on the histopathological severity [1]. Although liver biopsy has traditionally been considered the only reference method for evaluation of tissue damage, problem is that liver biopsy only gives a snapshot and not an insight into the dynamic changes during the process [31]. Moreover, it is invasive, difficult to accept, and susceptible to significant sampling variability and has a risk of severe complications [1]. Therefore, an ideal noninvasive surrogate marker or tests is urgently needed.

Several blood biomarkers and scores systems have been proposed for differential diagnosis of simple steatosis from NASH. Among the clinical and laboratory parameters, the best representative biomarker is cytokeratin-18 with 66% of Sen and 82% of Spe [12]. Besides, NAFIC score (AUROC = 0.851) were reported to be useful for diagnosing the early stage of NASH via the combination with blood markers such as Palekar's score [32, 33]. However, the use of laboratory indexes above is unusual and costly. Moreover, none of them are liver specific and their results may be influenced by changes in clearance and excretion of each individual parameter. Therefore, more longitudinally verifiable data are needed. It is known that the best-studied imaging modality was magnetic resonance elastography (MRE), demonstrating the high accuracy (AUROC = 0.93) for discriminating patients with NASH from those with simple steatosis, with 94% of Sen and 73% of Spe [34]. However, the group design utilized in this study lacked the clear histological diagnosis between NASH and not-NASH disease. To date, noninvasive tests have not been validated for diagnosing NASH because of the small number of studies.

SWE is an emerging noninvasive method based on shear waves implemented on the diagnostic ultrasound system to provide quantitative analysis of tissue stiffness. In the present study, we evaluated the performance of SWE on the diagnosis of NAFLD and compared it with the four serum fibrosis scores using histopathology as reference. The LSMs on SWE was proved to be a reliable method that can not only bring up potentially steatohepatitis hint, but also roughly determine the stage of liver fibrosis and would not been influenced by the presence of steatosis at the same time.

In the present study, we evaluated the performance of SWE in indentifying NASH in NAFLD patients and confirmed the good performance of SWE to diagnose NASH with an AUROC of 0.85 (0.73–0.92) at a cutoff of 7.55 kPa. To the best of our knowledge, this is the first study to evaluated SWE in a clinic concerned NASH, which was deemed to steatosis with lobular and portal area inflammation rather than minor inflammation.

As for staging fibrosis, dozens of noninvasive models composed of blood biochemical biomarkers were reported to be useful, including NFS [13], FIB-4 [14], APRI [25], enhanced liver fibrosis (ELF) [35] and CA index [36]. However, APRI and FIB-4 were initially established for patients with hepatitis C virus (HCV) infection or HIV/HCV coinfection. ELF and CA index need some special tests. In this study, the AUROC of SWE LSMs on detection of fibrosis is superior to those of four scoring systems (NFS, FIB-4, APRI and BARD). This is reasonable because some blood markers of the scoring systems would be affected by races and diets, whereas the SWE LSMs is directly reflect stiffness of the liver and guided by a higher frame-rate B-mode image that can yield a more accurate measurement. Transient elastography (TE) was initially shown to be reliable for assessing fibrosis in patients with chronic hepatitis C [15]. TE also was reported to be useful to assess fibrosis in patients with NAFLD [21, 37]. However, there were some limitations of TE. The first is its one-dimensional imaging that may fail to obtain reliable LSMs. Besides, TE has poor performance related to obesity, narrow intercostal space, and ascites [1]. SWE is a more accuracy method to staging fibrosis than TE. Ferraioli et al. showed that SWE was more accurate than TE in assessing significant fibrosis (≥ F2) in chronic hepatitis C [21]. Similarly, a recent meta-analysis studied for NAFLD patients [38] showed the pooled Sen and Spe for diagnosing F ≥ 2, F ≥ 3, and F = 4 disease, TE were 76% and 65%, 75% and 74%, 88% and 82%, respectively, SWE were higher than TE, were 85% and 94%, 90% and 92%, 100% and 86%, respectively.

As for NAFLD disease, some animal studies have reported that SWE is an efficient technique to differentiate NASH from less severe NAFL [39, 40]. However, limited human data were available on NAFLD. Samir AE et al.'s study showed that the use of SWE provided an AUROC of 0.77 (95% CI: 0.68, 0.86), with an optimal cutoff of 7.29 kPa (Sen = 91.4%, Spe = 52.5%) for ≥ F2 patients with a varied spectrum of liver diseases (NAFLD included) [41]. Garcovich et al. assessed SWE for diagnosing NASH in pediatric population and reported an AUROC of 0.96 to diagnose ≥ F2 with a cutoff value of 6.7 kPa (Sen = 87%, Spe = 96%) [42]. Arinc ozturk et al [43] demonstrated the higher threshold of 8.37 kPa than that of 7.65 kPa in this study to diagnose high risk NASH (F ≥ 2). This result may be accounted for different histopathological outcome markers or different patient samples. To date, apart from this study, no reliably diagnostic threshold to detect NASH in general population.

In the present retrospective study, the LSMs between the normal control group and NAFL group was no statistically difference (P = 1.0). Similarly, Suh et al. used SWE to study liver LSMs and demonstrated no statistical difference between the normal control group and NAFL group (P = 0.694) [44], suggesting that steatosis would not affect liver LSMs. Samir AE et al. also proved steatosis did not show any correlation with LSMs [41], consistent with our results. However, it cannot be ignored that the

influence of severe steatosis on LSMs, because fatty attenuation of severe steatosis may lead to SWE measurement failure [24]. Nevertheless, several literatures had inconsistent conclusions. In some transient elastography studies, steatosis has been reported to have an effect on TE LSMs [45–47].

One of the strengths of the study is that we consulted clinically concerned NAFLD classifications [11]: steatosis with minor inflammation is regarded as non-progressive and assigned to the NAFL group, rather than early NASH. So we can make a more clear diagnosis between NASH and non-NASH. Furthermore, we derived threshold to detect NASH and stage fibrosis. This result can be used for consensus establishment in clinical practice on thresholds or strategies for diagnosing NASH and differentiating fibrosis stage, or further trying to avoid liver biopsy. In addition, we evaluated in this study four fibrosis scoring biochemical systems in Chinese population. However, our study has some limitations such as limited sample size, bias of retrospective study, sampling error of needle biopsy, et al. Larger studies are needed to define the effect of these and other confounders and to establish SWE thresholds for various fibrosis stages in distinct diffuse liver disease.

Abbreviations

NAFLD nonalcoholic fatty liver disease

SWE shear wave elastography

NAFL nonalcoholic fatty liver

NASH nonalcoholic steatohepatitis

NFS NAFLD fibrosis score

FIB-4 fibrosis 4 calculator score

LSMs liver stiffness measurements

AUROC area under the receiver operating characteristics curve

TE transient elastography

TB total bile acid

DB direct bile acid

TG triglyceride

ALT alanine transaminase

AST aspartate transaminase

ALP alkaline phosphatase

LDH lactic dehydrogenase

BMI body mass index

SD standard deviations

Sen sensitivity

Spe specificity

PPV positive predictive value

NPV negative predictive value

95% CI 95% confidence interval

MRE magnetic resonance elastography

Declarations

Data sharing statement: The datasets generated and analysed during the current study are not publicly available due to ensure data privacy of the study participants but are available from the corresponding author on reasonable request.

Ethics approval and consent to participate: Informed consent was not required by the review board because patients did not need to be contacted for this retrospective data analysis. And patients' privacy is not included in this retrospective study. All experimental protocols were approved by Sichuan University. All methods were carried out in accordance with standards for reporting of diagnostic accuracy.

Conflict of interest: The authors have no financial conflicts of interest.

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Authors' contribution: Yan Luo had made substantial contribution to the conception and design of the work. And the main sponsor of the research. Jie Zhou collected and analyses the data, write the main manuscript text and prepared all figures and tables. Feng Yan and Jinshun Xu are responsible for major modification work. Qiang Lu provide statistical professional consultation. Xianglan Zhu give the histopathology assessment. Binyang Gao, Huan Zhang and Rui Yang provide software usage teaching, including R studio and programming statements.

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Figures

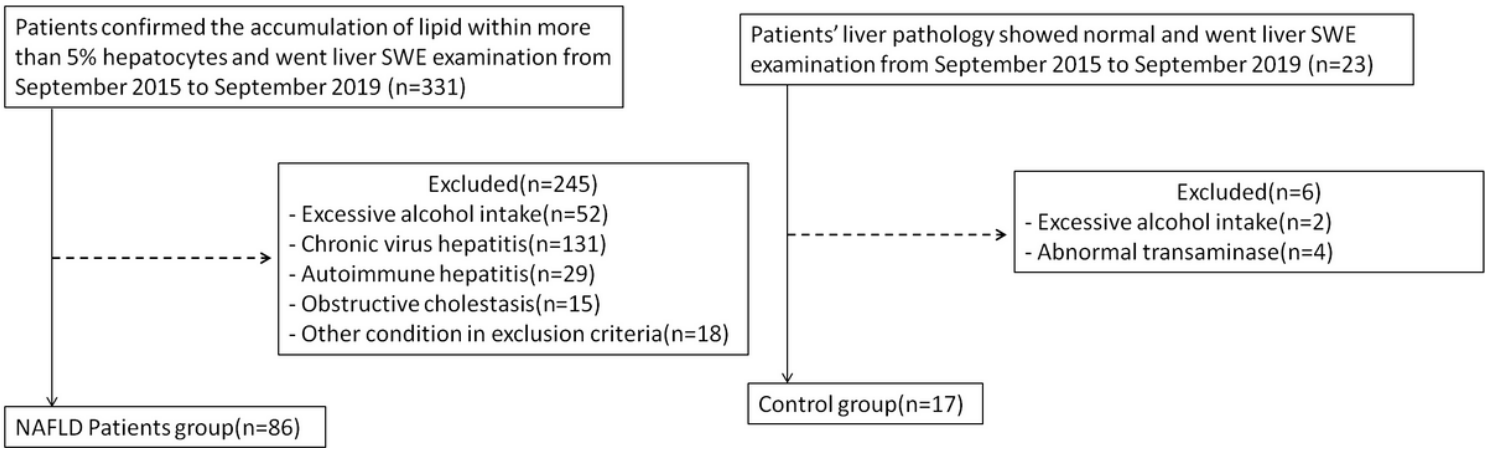

Figure 1

Flow chart of NAFLD patients and normal control group. Note: abbreviation SWE, real-time shear wave elastography; NAFLD, nonalcoholic fatty liver disease.

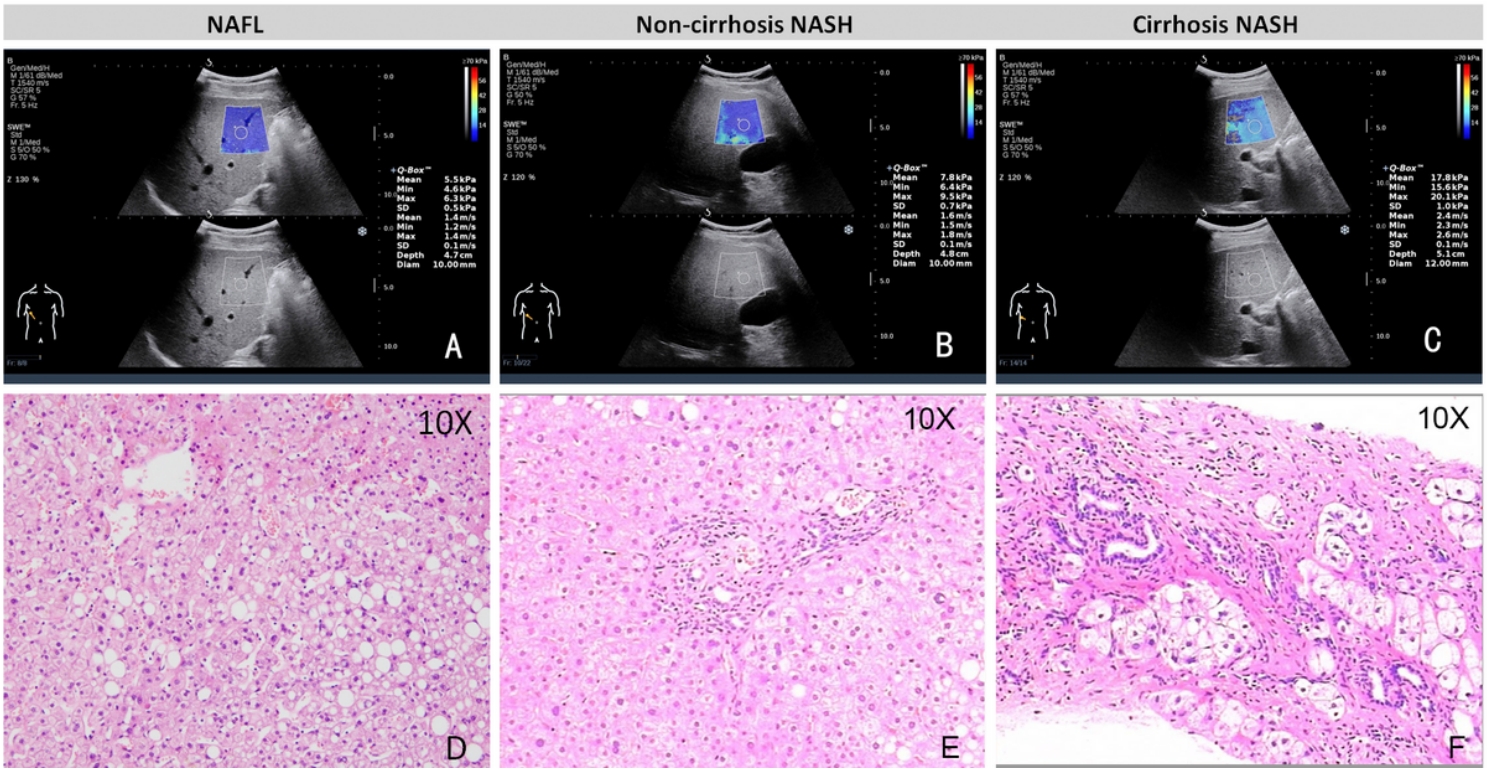

Figure 2

Results of shear wave elastography images (top row) and HE stain sections (bottom row) in patients with NAFLD having simple steatosis (A and D), non-cirrhosis NASH (B and E), and cirrhosis NASH (C and F). The mean liver stiffness was 5.5 kPa, 7.8 kPa, and 13.8 kPa, respectively.

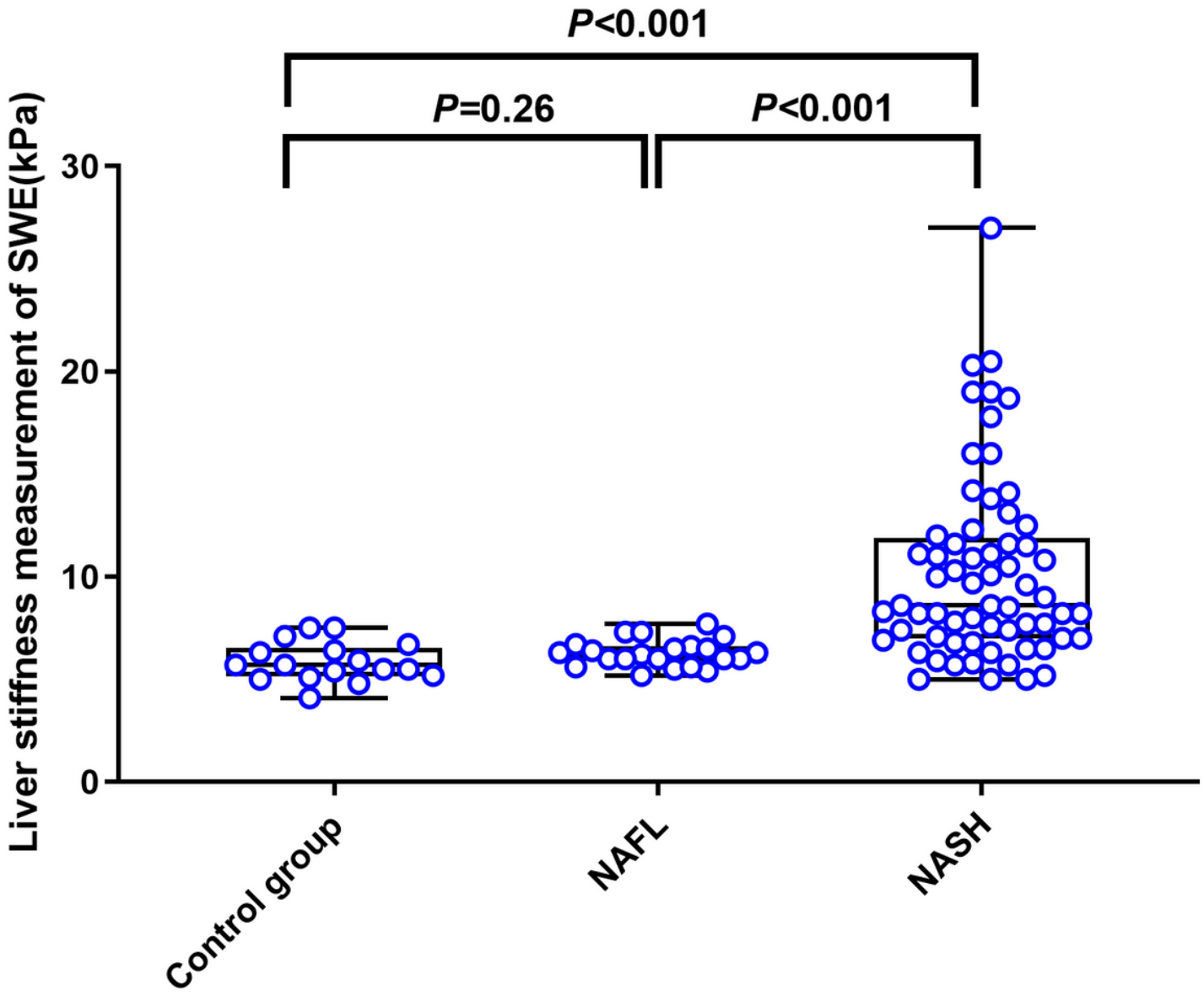

Figure 3

Distribution liver stiffness measurements (LSMs) by SWE of the normal control, NAFL, and NASH group. The LSMs (±SD) of NASH group (10.2±4.5kPa) were higher than both normal control (6.1±0.9 kPa) and NAFL group (6.2±0.7 kPa) (P<0.001 for all). No statistical difference was found for LSMs as compared with normal control and NAFL group (P 0.26).

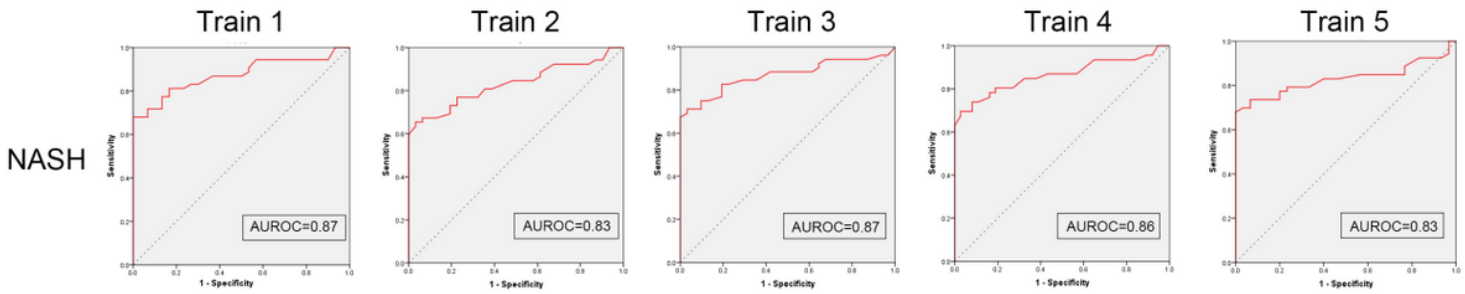

Figure 4

Graphs show 5-fold cross validation of ROC curves for diagnosing NASH by SWE in train sets, the mean AUROC=0.85.

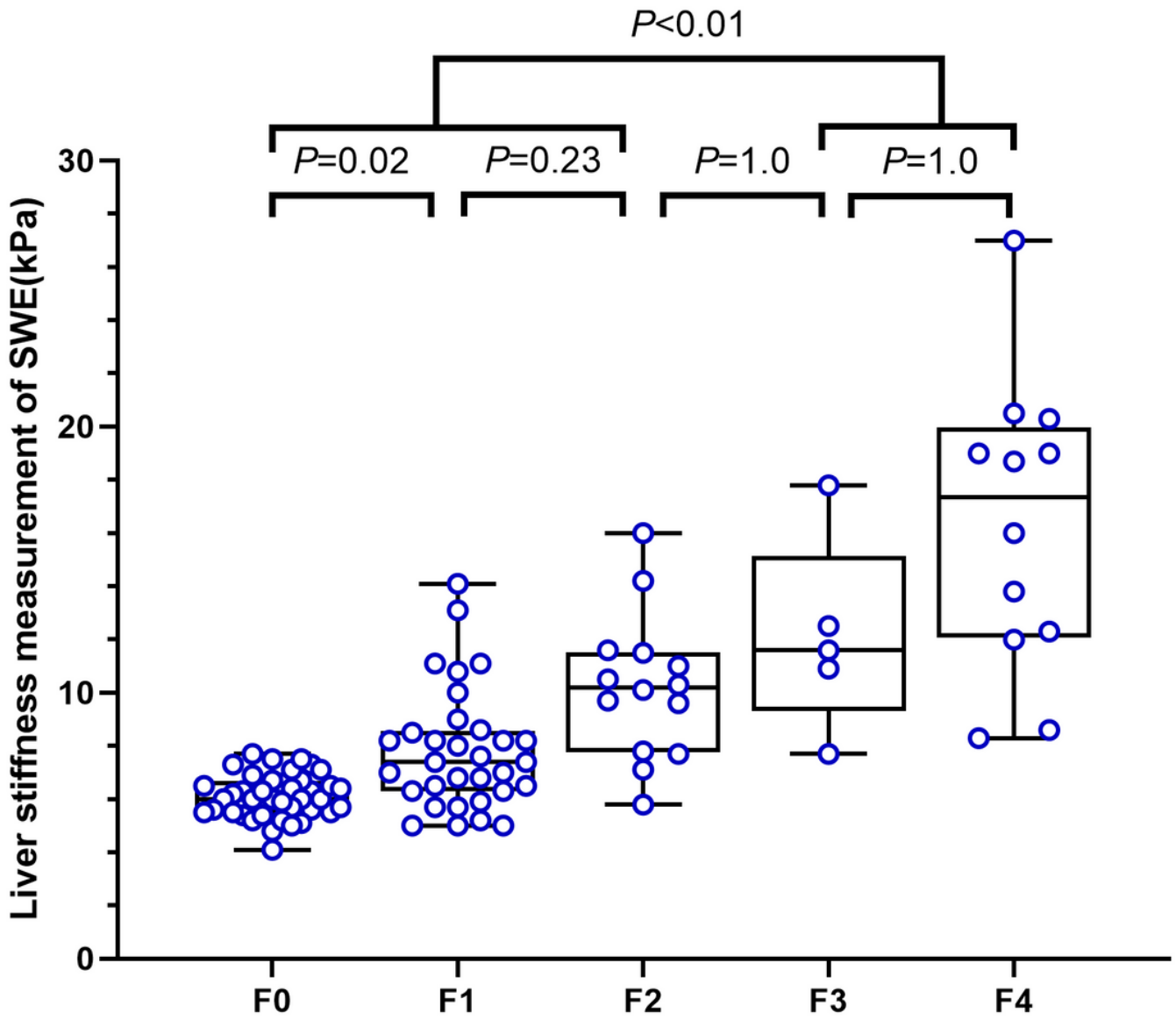

Figure 5

The diagnostic performance of the LSMs on SWE according to fibrosis stage. The mean LSMs (±SD) of NAFLD patients from F0 to F4 were 6.1±0.9, 7.8±2.3, 10.3±2.7, 12.2±3.6 and 16.3±5.5kPa, respectively. The LSMs of non-advanced fibrosis (F0-2) and advanced types (F3-4) had statistic difference (P□0.01). However, except for F0 and F1, the LSMs between two adjacent fibrosis stages had no statistic difference (P□0.05 for all).

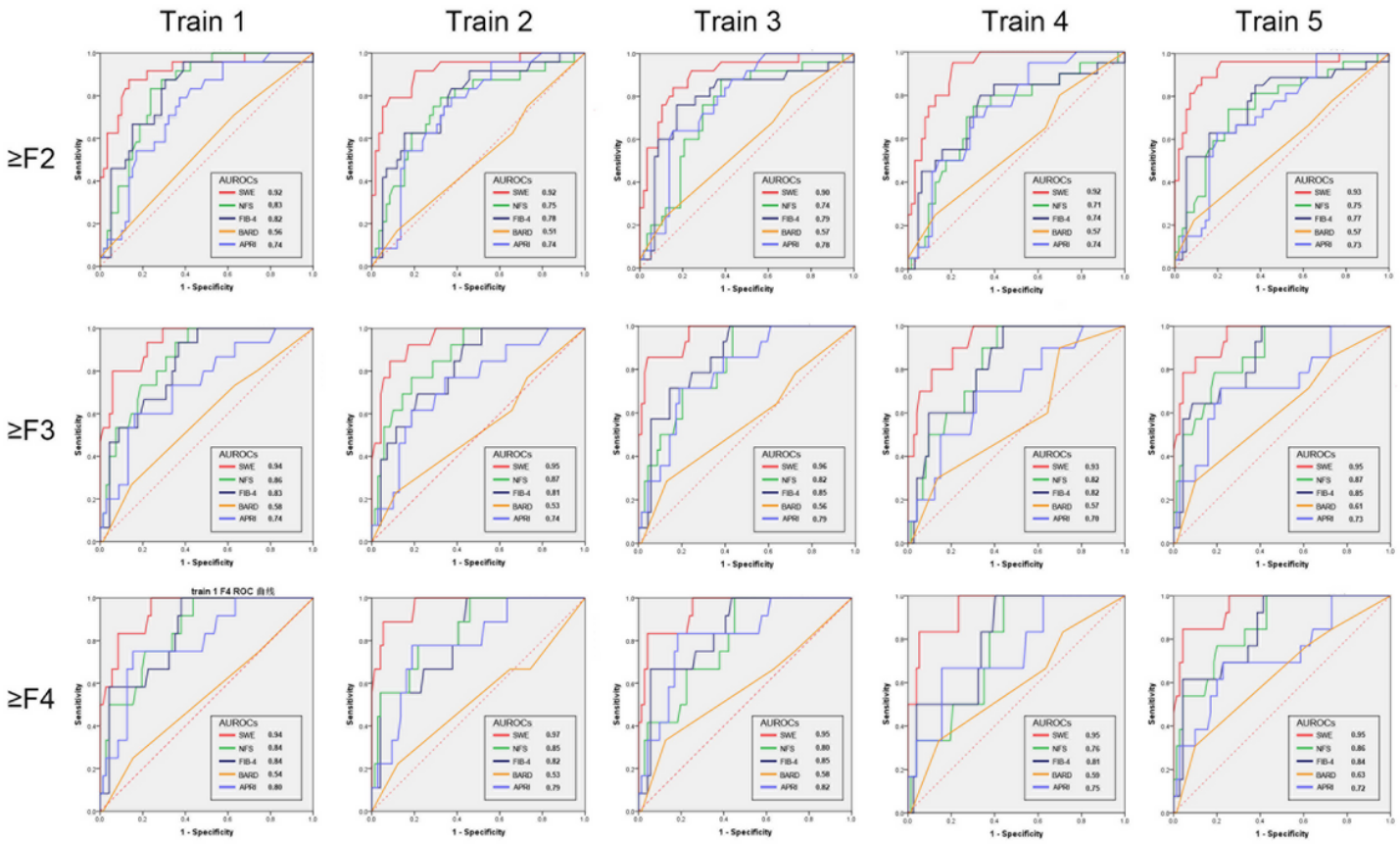

Figure 6

The ROC curves of SWE and four serum fibrosis scores (NFS, FIB-4, APRI and BARD scores) for staging fibrosis in train sets of 5-fold cross validation.

Supplementary Files

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