Turk J Gastroenterol *2015; 26: 408-16*

The development of a clinical score for the prediction of nonalcoholic steatohepatitis in patients with nonalcoholic fatty liver disease using routine parameters

LIVER

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ABSTRACT

Background/Aims: To develop a clinical score for the prediction of nonalcoholic steatohepatitis (NASH) using routine parameters in patients with nonalcoholic fatty liver disease who had abnormal liver function tests and/or fatty liver detected by ultrasonography.

Materials and Methods: To identify parameters associated with the presence of NASH by evaluating anthropometric characteristics and routine biomarkers of 82 patients with histologically proven NAFLD and to develop a clinical score for predicting NASH according to the area under the curve of receiver operating characteristics (AUC) of parameters.

Results: Four parameters [alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), C-reactive protein (CRP), and apolipoprotein B (ApoB)/apolipoprotein A1 (ApoA1) ratio] were significantly linked to NASH. The AUROCs of ALT, GGT, CRP, and ApoB/ApoA1 ratio for the prediction of NASH were 0.829, 0.892, 0.708, and 0.712, respectively. The AUROC of the combined clinical score for the prediction of NASH was 0.904 (95% CI, 0.885–1.002) at a cut-off of 3.8 points with a sensitivity of 90.2%, specificity of 87.0%, positive predictive value of 88.3%, and negative predictive value of 91.5%.

Conclusion: In the present study, a clinical score was developed for the noninvasive prediction of NASH; the score was based on a combination of routine biochemical parameters and was useful in distinguishing NASH from NAFLD.

Keywords: Nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, biomarker, clinical score

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is currently the leading cause of liver disease, with an estimated prevalence of 20%–35% in developed countries, 20%–30% in Western populations (1), and 11%–15% in Chinese populations (2). NAFLD is the pathological accumulation of fat in hepatocytes that is not associated with alcohol intake (3). Its development is strongly associated with obesity, insulin resistance, and other components of the metabolic syndrome (4,5). NAFLD includes a spectrum of abnormalities, ranging from simple liver steatosis (NASS) to nonalcoholic steatohepatitis (NASH) (6). Although most patients with NAFLD follow a benign course, a subset of patients have an increased risk of progression to end-stage liver disease and exhibit an increased liver-related mortality rate (7). NASH is characterized by accompanying apoptosis, inflammation, and fibrosis, representing the progressive form of NAFLD and may further evolve to cryptogenic cirrhosis (8). The natural history of NAFLD shows that histopathological progression occurs in 32%–37% of patients over 3–6 years; up to 12% of patients may progress to cirrhosis over 8–10 years (9). Although ultrasonography is used extensively in the diagnosis of NAFLD, this method is not effective in differentiating between NASS and NASH (10). Liver biopsy is still considered to be the best available standard for assessing the extent of inflammation and fibrosis of the liver in NAFLD; however, it

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Received: October 17, 2013 **Accepted:** February 18, 2014 **Available Online Date:** July 24, 2015

is an invasive and costly procedure and is associated with sampling variability as well as high intra- and inter-pathologist variability (11). Most patients with NAFLD do not know whether they have NASH unless a liver biopsy is performed; thus, it is not detected early and managed appropriately. Therefore, there is a need to develop noninvasive methods to identify patients who have NASH or are at a risk of NASH. Several studies describing noninvasive prediction models have been published; however, the parameters used in these studies are either too extensive or are not readily available in clinical practice. The aim of the present study was to identify risk factors for NASH by assessing clinical features and biochemical markers and to establish a clinical score for the prediction of NASH in NAFLD patients using simple routine parameters before NAFLD progresses to the stage of advanced fibrosis.

MATERIALS AND METHODS

Patients

The clinical data of 322 patients who were admitted to the Zhenjiang Third Hospital from July 2006 to March 2013 for abnormal liver function tests and/or fatty liver detected by ultrasonography were retrospectively analyzed. Inclusion criteria comprised the absence of significant alcohol abuse defined by an average daily consumption of alcohol of >20 g/day in males and >10 g/day in females (12); negative tests for the presence of hepatitis B surface antigen and antibodies to hepatitis C virus; no evidence of use of hepatotoxic drugs; and no autoimmune liver disease. A total of 104 patients who fulfilled the inclusion criteria underwent the measurement of anthropometric characteristics, blood testing, and liver biopsy. All patients included in the present retrospective study provided their written informed consent prior to liver biopsy. The baseline anthropometric characteristic and biochemical markers of all patients were collected after their admission. According to the histological characteristics, the patients with histologically proven NAFLD were divided into the NASS and NASH groups. The former included nonalcoholic patients with simple steatosis, while the latter included patients with NASH. A total of 82 patients who had complete clinical data, eligible liver biopsy specimens, and histological diagnosis were included in the analysis (Figure 1). The present study was conducted in accordance with the Declaration of Helsinki, and it was approved by the Ethical Committee of the Jiangsu University School of Medicine and conformed to the National Institute of Health guidelines regarding clinical studies. Informed consent was obtained from all patients.

Clinical assessment

Anthropometric characteristics, including age, gender, body weight, height, and body mass index (BMI), were measured using automated instruments, while patients were barefoot and wore light clothing. Systolic and diastolic blood pressures were measured on the right upper arm using an electronic blood pressure manometer. Waist circumference was measured at the halfway point between the lowest part of the ribs and the iliac crest while the patient was in a standing position. BMI was calculated as weight (kg) divided by height (m^2).

Biochemical analyses

Blood samples were collected from the antecubital vein after an overnight fast. Biochemical markers, including fasting blood glucose (FBG), glycosylated hemoglobin (HbA1c), total cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL), apolipoprotein A1 (ApoA1), apolipoprotein B (ApoB), serum total bilirubin (TBIL), direct bilirubin (DBIL), indirect bilirubin (IBIL), albumin (ALB), globulin (GLB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AKP), gamma-glutamyl transferase (GGT), lactate dehydrogenase (LDH), serum lipid, C-reactive protein (CRP), and serum uric acid and ferritin levels, were measured according to the manufacturer's instruction. Serum tests were performed using an automatic biochemistry analyzer with standard laboratory methods, with the normal reference range of 0–40 IU/L for ALT and 0–42 IU/L for GGT. CRP was measured by immunoturbidimetry with a normal reference range of 0–7 mg/dL and an analytical sensitivity of 0.5 mg/dL. ApoB and ApoA1 concentrations were measured by particle-enhanced immunonephelometry with the normal reference ranges of 0.8–1.1 g/L and 1.2–1.6 g/L, respectively.

Ultrasonographic assessment

Abdominal ultrasonography was performed by radiologists who were experienced in diagnosing fatty liver disease. The diagnosis of fatty liver was based on hepatorenal echo contrast, liver brightness, deep-echo attenuation, and vascular blurring (13). An ultrasound device with a 3.5-MHz convex-type transducer (EUB-8500 scanner; Hitachi Medical Corporation, Tokyo, Japan) was used.

Histological evaluation

All patients underwent an ultrasound-guided percutaneous liver biopsy using a 16-gauge needle (Hepafix; Braun, Germany) under local anesthesia. Liver biopsy specimens were fixed in 10% buffered formalin, embedded in paraffin, and stained with hematoxylin and eosin, Masson-trichrome, and/or reticulin stains. Only liver fragments that were at least 1.5 cm in length and included eight portal tracts were considered to be appropriate for histological assessment. The presence of steatotic hepatocytes at a concentration of >5% in a liver tissue section was accepted as the histological criterion for the diagnosis of NAFLD (14). NAFLD activity score (NAS) was used to diagnose NAFLD (15). NAS represents the sum of the scores for steatosis (0–3), lobular inflammation (0–3), and ballooning (0–2); NAS scores range from 0 to 8. NASH is defined as an NAS score of ≥5. NAS scores of 0–2 are not considered to be diagnostic of NASH; scores of 3 to 4 are considered "borderline," and scores of 5 to 8 are considered to be diagnostic of NASH. Patients were divided into the following two groups according to their histological diagnosis: the NASH group (NAS>5) and the NASS group (NAS<3).

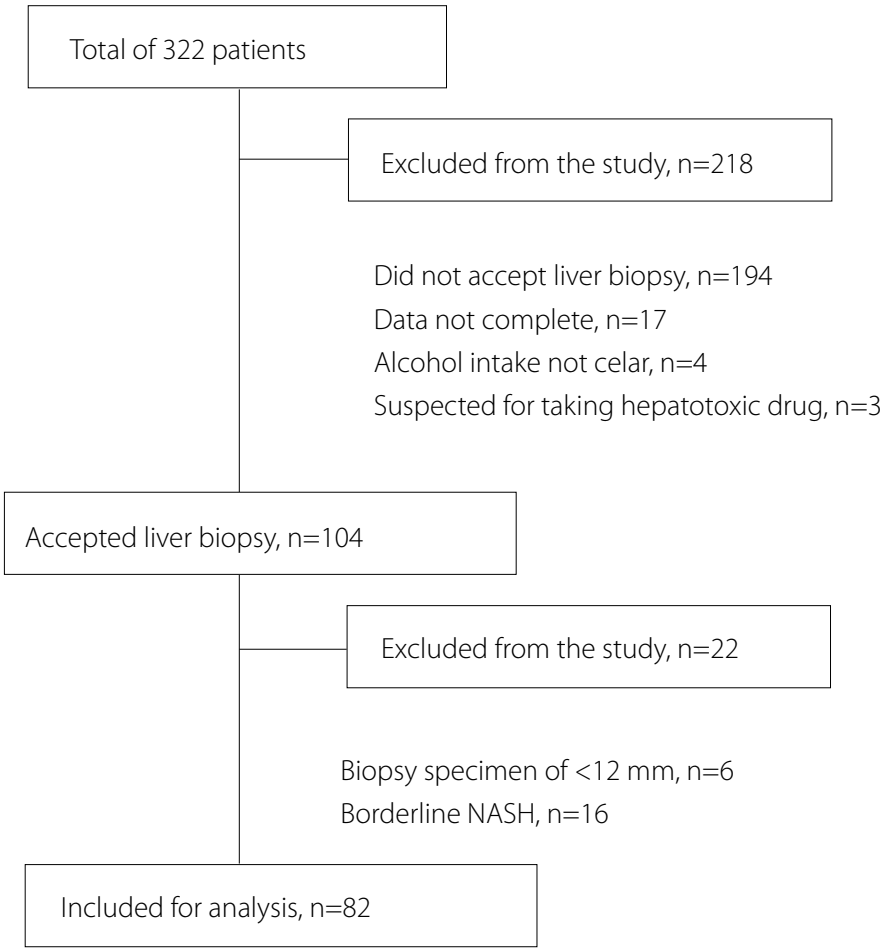

Figure 1. Flow chart of the selection of 82 patients included in the present patients analysis.

Statistical analysis

Data were expressed as mean±standard deviation (SD) for continuous variables and as frequency with percentage for categorical variables. Continuous variables were compared using Student's *t*-test or the Mann–Whitney U test, and categorical variables were compared using Pearson's chi-square test. The independent risk factors related to NASH were selected using a binary (NASH=1; NASS=0) logistic stepwise regression model. The area under the receiver operating characteristic (ROC) curve (AUC) was used to measure the performance of the clinical score in predicting the NASH of patients with NAFLD. AUC ranges from 0 to 1. An AUC of >0.7 is generally considered to be useful, and an AUC between 0.8 and 0.9 indicates good diagnostic accuracy. The sensitivity (SE), specificity (SP), positive predictive value (PPV), and negative predictive value (NPV) were calculated according to the most predictive cut-off value that had the best discrimination ability to predict NASH. For all analyses, p values of <0.05 were considered to be statistically significant. Statistical analyses were performed using SPSS software version 16.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Patient characteristics

A total of 82 patients with histologically proven NAFLD (54 with NASS; 28 with NASH) were included in the present study (Figure 1). The mean ages of the patients were 45.44±14.34 years and 47.47±13.16 years, respectively. Of the 82 patients, there were 32 (59.2%) men and 22 (40.7%) women in the NASS group, and there were 17 (60.2%) men and 11 (39.2%) women in the NASH group; the distribution of gender was not significantly different between the groups (p=0.54). Compared with the NASS group, the NASH group had higher mean systolic blood pressure (139.32±10.92 vs. 134.9±89.65 mmHg), diastolic blood pressure (81.93±9.21 mmHg vs. 78.74±8.89 mmHg), FBG levels (6.02±1.75 mmHg vs. 5.57±1.37 mmol/L), HbA1 levels (6.03±0.74% vs. 5.95±1.17%), WC (92.28±6.64 cm vs. 89.23±8.09 cm), body weight (75.46±6.88 kg vs. 73.35±6.70 kg), and BMI (27.97±1.55 kg/m^2 vs. 26.02±1.71 kg/m^2). The proportion of patients with hypertension (35.7% vs. 29.6%), hyperglycemia (28.5% vs. 18.5%), central obesity (71.4% vs. 53.7%), and exces-

sive weight (82.1% vs. 70.3%) were higher in the NASH group than in the NASS group, but only BMI was significantly different between the two groups (p=0.02; Table 1).

Comparison of biochemical characteristic between the NASS and NASH groups

As shown in Table 2, the univariate analysis using Student's *t*-test showed that serum levels of TG, LDL, LDL/HDL, ApoB, and ApoB/ApoA1 ratio were higher in the NASH group than in the NASS group (p=0.023, 0.002, 0.012, 0.000, and 0.002, respectively), but the levels of serum TC, HDL, and ApoA1 were not significantly different between the NASH and NASS groups (p=0.087, 0.370, and 0.674, respectively). The univariate analysis of the liver function index showed that the serum levels of ALT and AST, AST/ALT ratio, and levels of AKP, GGT, and LDH were significantly higher in the NASH group than in the NASS group (p=0.000, 0.014, 0.002, 0.024, 0.000, and 0.025, respectively), whereas the levels of serum TBIL, DBIL, IBIL, ALB, and GLB were not significantly different (p=0.0.254, 0.815, 0.150, 0.654, and 0.090, respectively) between the two groups. Other serum biochemical markers (CRP and ferritin levels) were also significantly different (p=0.003 and 0.006, respectively) between the NASH and NASS groups by the univariate analysis. However, serum uric acid levels were not significantly different (p=0.163) between the two groups. On the basis of the univariate analysis, significant variables included TG, LDL, LDL/HDL, ApoB, ApoB/ApoA ratio, ALT, AST, AST/ALT, AKP, GGT, LDH, CRP, ferritin, and BMI (Table 1).

Risk factors for predicting the presence of NASH by the logistic regression analysis

With the presence of NASH as the dependent variable, variables that were significantly different between the NASH and NASS groups from the univariate analysis (p<0.05) included TG, LDL, LDL/HDL and ApoB levels, ApoB/ApoA1 ratio, ALT, AST, AST/ALT, AKP, GGT, LDH, CRP and ferritin levels, and BMI as independent variables. A binary (NASH=1; NASS=0) logistic regression analysis was performed by the logistic stepwise regression analysis. At the final step, four variables (ALT, GGT, CRP, and ApoB/ApoA1 ratio) were introduced into the model as significant predictors of the presence of NASH (for ALT: OR=1.028, 95% CI=1.009–1.047, p=0.004; for GGT: OR=1.016, 95% CI=1.004–1.027, p=0.009; for CRP: OR=1.472, 95% CI=1.146–1.892, p=0.002; and for ApoB/ApoA1 ratio: OR=39.131, 95% CI=29.223–136.495, p=0.008). This revealed that ALT, GGT, CRP, and ApoB/ApoA1 ratio were independent risk factors for predicting the presence of NASH. Mean serum levels of these four variables were higher in the NASH group than in the NASS group (as shown in Table 3 and Figure 2).

The accuracy of four risk factors predicting NASH alone and calculation of clinical scores

On the basis of the four risk factors identified by the binary logistic stepwise regression analysis, ROC curves for ALT,

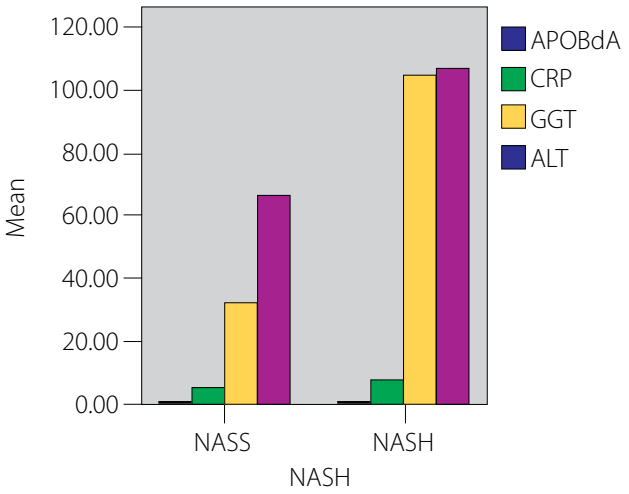

Figure 2. Comparison of the levels of four parameters between the non-alcoholic steatohepatitis (NASH) group and the simple liver steatosis (NASS) group. APOBdA = the ratio of apolipoprotein B to apolipoprotein A1; CRP: C reactive protein; GGT: gamma-glutamyl- transferase; ALT: alanineaminotransferase

Table 1. Comparison of anthropometric characteristics between the nonalcoholic simple liver steatosis (NASS) group and the nonalcoholic steatohepatitis (NASH) group

Characteristic	NASS group, n=54	NASH group, n=28	p
Age (years)	45.44±14.34	47.17±13.17	0.59
Male/Female, n (%)/n (%)	32 (59.2)/22 (40.7)	17 (60.7)/11 (39.2)	0.54
Systolic blood pressure (mmHg)	134.9±89.65	139.32±10.92	0.13
Diastolic blood pressure (mmHg)	78.74±8.89	81.93±9.21	0.25
Hypertension			
Yes, n (%)	16 (29.6)	10 (35.7)	0.37
No, n (%)	38 (70.3)	18 (64.2)	
Fasting blood glucose (mmol/L)	5.57±1.37	6.02±1.75	0.19
HbA1c (%)	5.95±1.17	6.03±0.74	0.74
hyperglycemia			
Yes, n (%)	10 (18.5)	8 (28.5)	0.44
No, n (%)	44 (81.5)	20 (71.4)	
Waist circumference (cm)	89.23±8.09	92.28±6.64	0.09
Central obesity			
≥80 for female/90 for male, n (%)	29 (53.7)	20 (71.4)	0.16
≤80 for female/90 for male, n (%)	25 (46.3)	8 (28.5)	
Weight (kg)	73.35±6.70	75.46±6.88	0.18
Height (m)	1.68±0.06	1.67±0.07	0.66
Body mass index (kg/m^2)	26.02±1.71	27.97±1.55	0.02
Overweight (Yes/No)			
Yes, n (%)	38 (70.3)	23 (82.1)	0.22
No, n (%)	16 (29.6)	5 (17.8)	

HbA1c=glycosylated hemoglobin

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Table 2. Comparison of baseline biochemical markers between the nonalcoholic simple steatosis (NASS) group and the nonalcoholic steatohepatitis (NASH) group

Characteristics	NASS group	NASH group	p
Lipid profile			
Triglycerides (mmol/L)	1.57±0.59	2.29±1.55	0.023
Total cholesterol (mmol/L)	4.28±0.91	4.73±1.44	0.087
High-density lipoprotein (mmol/L)	1.44±0.32	1.51±0.24	0.370
Low-density lipoprotein (mmol/L)	2.17±0.72	2.78±0.98	0.002
LDL/HDL ratio	1.49±0.33	1.76±0.62	0.012
ApoA (g/L)	1.26±0.27	1.29±0.44	0.674
ApoB (g/L)	0.96±0.26	1.20±0.27	0.000
ApoB/ApoA1 ratio	0.78±0.21	0.98±0.29	0.002
Liver function index			
Alanine transaminase (IU/L)	66.50±34.27	106.32±34.43	0.000
Aspartate transaminase (IU/L)	54.09±31.70	70.96±22.63	0.014
AST/ALT ratio	0.86±0.25	0.69±0.16	0.002
Alkaline phosphatase (IU/L)	87.70±41.63	113.46±58.37	0.024
Gamma-glutamyl transferase (IU/L)	32.24±17.02	105.00±75.67	0.000
Lactate dehydrogenase (IU/L)	180.15±52.04	207.43±49.85	0.025
Albumin (g/L)	42.02±2.71	42.34±3.82	0.654
Globumin (g/L)	31.87±4.36	33.58±4.10	0.090
Total bilirubin (μmol/L)	13.81±4.66	15.09±4.95	0.254
Direct bilirubin (μmol/L)	3.66±1.79	3.76±1.63	0.815
Indirect bilirubin (μmol/L)	10.15±3.42	11.33±3.62	0.150
Other biochemical markers			
C-reactive protein (mg/L)	5.54±2.26	8.07±3.89	0.003
Ferritin (mg/L)	383.27±183.34	569.71±313.63	0.006
Serum uric acid (μmol/L)	327.35±72.86	352.91±87.19	0.163

Values are expressed as mean±SD. LDL: low-density lipoprotein; HDL: high-density lipoprotein; ApoA1: apolipoprotein A1; ApoB: apolipoprotein B; AST: aspartate aminotransferase; ALT: alanine aminotransferase

GGT, CRP, and ApoB/ApoA1 ratio alone were plotted to verify the accuracy of predicting NASH and to determine cut-off points with the best performance for the prediction of NASH. The AUROCs for ALT, GGT, CRP, and ApoB/ApoA1 ratio alone predicting NASH were 0.829, 0.892, 0.708, and 0.712, respectively. It was observed that the AUROCs of ALT and GGT was larger than those of CRP and ApoB/ApoA1 ratio; the cut-offs for ALT, GGT, CRP and ApoB/ApoA1 ratio for predicting NASH were ALT of ≥75 IU/L, GGT of ≥55 IU/L, CRP of ≥5.6 mg/L, and ApoB/ApoA1 ratio of ≥0.8. The SE, SP, PPV, and NPV of ALT, GGT, CRP, and ApoB/ApoA1 ratio at their respective cut-off values were 82.1%, 74.8%, 82.3%, and 71.2% for ALT; 85.7%, 74.1%, 77.5%, and 81.0% for GGT; 71.4%, 59.3%, 68.8%, and 75.3% for CRP; and 78.6%, 73.0%, 71.4%, and 75.3% for ApoB/ApoA1 ratio. To construct clinical scores using the results of the multiple logistic regression analyses and AUROCs, clinical scores (as integers) were assigned to the four independent risk factors. By assigning one point each to CRP and ApoB/ApoA1 ratio and two points each to ALT and GGT according to the size of the AUROCs of the variable, the final clinical score ranged from a minimum of zero points to a maximum of six points. The points were given to each factor as follows: for ALT, AUROCs=0.829 (95% CI, 0.737–0.921), >0.8, two points; for GGT, AUROCs=0.892 (95% CI, 0.817–0.967), >0.8, two points; for CRP, AUROCs=0.708 (95% CI, 0.588–0.828), <0.8, one point; for ApoB/ApoA1 ratio, AUROCs=0.712 (95% CI, 0.590–0.833), <0.8, one point (as presented in Table 4 and Figure 3).

Table 3. Four independent risk factors associated with the presence of nonalcoholic steatohepatitis

Variable	B	SE	Wald χ^2	p	OR	95% CI
ALT	0.028	0.009	8.495	0.004	1.028	1.009–1.047
GGT	0.015	0.006	6.789	0.009	1.016	1.004–1.027
CRP	0.387	0.128	9.151	0.002	1.472	1.146–1.892
ApoB/A1 ratio	4.145	1.568	6.988	0.008	63.131	29.2–136.46

Data were calculated using logistic stepwise regression analyses.
B: regression coefficient; SE: standard error; OR: odds ratio; 95% CI: 95% confidence interval; ALT: alanine aminotransferase; GGT: gamma-glutamyl transferase; CRP: C-reactive protein; ApoB/A1 ratio: ratio of apolipoprotein B to apolipoprotein A1

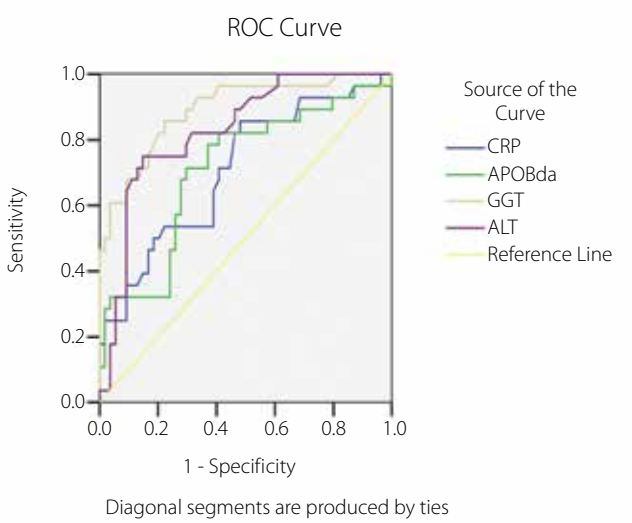

Figure 3. Receiver operator characteristic curves (ROC) of gamma-glutamyl transferase (GGT), alanine aminotransferase (ALT), C-reactive protein (CRP) and apolipoprotein B/apolipoprotin A1 ratio (APOBdA) in the prediction of NASH. A comparison of the four parameters for the prediction of NASH using ROC is shown. An area under the ROC (AUC) of 1.0 is characteristic of an ideal test, whereas an AUC of ≤0.5 represents a test with no diagnostic value.

The performance of clinical score in predicting the presence of NASH

To assess the accuracy of the clinical score, it was applied to all patients in the study, and its ROC curve was plotted; SE, SP, NPV, and PPV were then calculated at different scores ranging from zero to six. The AUC of the ROC curve was 0.904 (95% CI, 0.885–1.002); when two points were used as

Table 4. Predictive accuracy and optimal cut-off values of each factor for nonalcoholic steatohepatitis prediction

Variable	AUC	95% CI	cut-off	SE (%)	SP (%)	PPV (%)	NPV (%)
CRP	0.708	0.588–0.828	5.6	71.4	59.3	68.8	75.3
ApoB/ApoA1	0.712	0.590–0.833	0.8	78.6	73.0	71.4	74.4
ALT	0.829	0.737–0.921	75.0	82.1	74.8	82.3	71.2
GGT	0.892	0.817–0.967	55.0	85.7	74.1	77.5	81.0

AUC: area under the receiver operating characteristic curve; 95% CI=95% confidence interval; SE: sensitivity; SP: specificity; PPV: positive predictive value; NPV: negative predictive value; CRP: C-reactive protein; ApoB: apolipoprotein B; ApoA1: apolipoprotein A1; ALT: alanine aminotransferase; GGT: gamma-glutamyl transferase

Table 5. Accuracy of the clinical score for predicting the presence of nonalcoholic steatohepatitis (NASH)

Clinical score	Patients, n (%) n=82	NASH, n (%) n=28	SE (%)	SP (%)	PPV (%)	NPV (%)
0	22 (26.8)	2 (7.1)	99.2	37.8	54.2	97.6
1	6 (7.3)	3 (10.7)	97.3	44.4	65.5	96.4
2	15 (18.3)	4 (14.4)	94.4	51.4	71.4	95.5
3	19 (23.0)	6 (21.4)	93.9	82.2	85.8	93.5
4	14 (17.0)	7 (25.0)	88.4	90.4	92.4	87.8
5	5 (6.1)	5 (17.8)	52.1	92.6	94.7	79.6
6	3 (3.5)	1 (3.6)	24.3	98.8	96.2	70.2

The clinical score was induced by assigning two points to ALT and GGT and assigning one point to both CRP and ApoB/ApoA1 ratio, according to their area under the curve of the receiver operating characteristic curve for predicting NASH. Clinical score ranged from a minimum of zero points to a maximum of six points. NASH: nonalcoholic steatohepatitis; SE: sensitivity; SP: specificity; PPV: positive predictive value; NPV: negative predictive value

the cut-off value, SE was 94.4% and SP was 51.4%, and NASH could be ruled out. When five points were used as the cut-off value, SE was reduced to 52.1%, SP increased to 92.6%, and NASH could be considered. When 3.8 was used as the cut-off value, SE was 90.2%, SP was 87.0%, PPV was 88.3%, and NPV was 91.5%. This clinical score had the most optimal performance for predicting NASH (Table 5, Figure 4).

DISCUSSION

Nonalcoholic fatty liver disease covers a disease spectrum ranging from NASS to NASH (i.e., histological necroinflammation). NASH often leads to fibrosis, which can progress to end-stage liver diseases such as cirrhosis and hepatocellular carcinoma (16). Thus, it is important to distinguish NASH from NAFLD for its early management before NASH progresses to end-stage liver disease. Ultrasonography is extensively used to identify fatty liver, but it cannot differentiate NASH from NASS; although liver biopsy is still the best method for diagnosing NASH, its application is limited in clinical practice because of its disadvantages, including its invasiveness, sampling variability, and high intra- and interpathologist variability (17). Therefore, it is necessary to develop a noninvasive method that can be used instead of liver biopsy to reach a definite diagnosis of NASH.

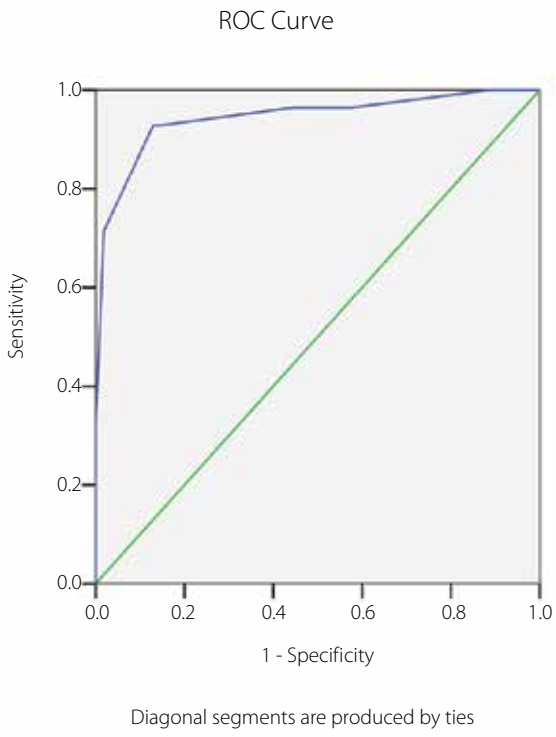

Figure 4. The receiver operating characteristic (ROC) curve of the clinical score constructed for the prediction of NASH. The area under the ROC curve is 0.904 (95% CI, 0.885–1.002); when 3.8 points is used as a cut-of value, the sensitivity and speciicity are 90.2% and 87.0%, respectively.

We evaluated the anthropometric characteristics and serum biochemical markers of 82 patients with NAFLD to construct a clinical score for the prediction of NASH. A univariate analysis and binary logistic regression analysis identified a total of four biochemical parameters: ALT, GGT, CRP, and ApoB/ApoA1 ratio. In all the parameters representing anthropometric characteristics, only BMI showed a statistically significant difference in the univariate analysis; however, it was removed following the logistic regression analysis, suggesting that the results of our study do not show a correlation between BMI and the presence of NASH. Other anthropometric characteristics, such as age, gender, waist circumference, body weight, height, blood pressure and the percentage of excessive weight, hyperglycemia, and hypertension, were not significantly different even in the univariate analysis.

In our study, ApoB/ApoA1 ratio was the only variable selected among all of the measures of serum lipid levels. As individual parameters, our study showed that ApoB/ApoA1 ratio at a cut-off of 0.8 had an SE of 78.6%, an SP of 73.0%, and an AUC of 0.712 (95% CI, 0.590–0.833) for predicting NASH in patients with NAFLD. A study by Young et al. (18) reported that ApoB/ApoA1 ratio was associated with NAFLD prevalence in nondiabetic subjects independent of obesity and other metabolic components, and NAFLD risk increased as ApoB/ApoA1 ratio increased; however, to date, no study has been

published regarding the relationship between ApoB/ApoA1 ratio and the presence of NASH. ApoA1 is the major apolipoprotein associated with HDL, whereas ApoB is present in non-HDL lipoprotein, which includes very low-density lipoprotein, intermediate-density lipoprotein, and LDL (19). Therefore, the elevation of ApoB/ApoA1 ratio implies that non-HDL levels are increasing and/or HDL levels are decreasing. Elevated non-HDL levels can lead to the accumulation of lipids within hepatocytes; the resulting increase in free fatty acid levels causes lipotoxicity and lipid peroxidation, impairing hepatocytes and resulting in the development of NASH (20,21).

Regarding other serum biochemical markers, our study showed that only CRP level was linked to the presence of NASH, whereas FBG, HbA1, ferritin and serum uric acid levels were not correlated with NASH. CRP is an acute phase reactant protein that may reflect the inflammatory status of hepatocytes. Yoneda et al. reported that CRP levels were significantly elevated in patients with NASH compared with those in patients with NASS, and serum CRP levels were significantly increased in patients with steatohepatitis compared with those in controls. ROC curve analysis revealed that serum CRP levels of >3 mg/L had an SE of 73% and an SP of 68% for NAFLD, suggesting that CRP may be a biological feature that distinguishes NASH from simple steatosis. Thus, CRP is an important independent predictive factor for the diagnosis of NASH (22). Our study showed that serum CRP level at a cut-off of 5.6 mg/L had an SE of 71.4%, SP of 59.3%, and AUC of 0.82 (95% CI: 0.78–0.88) for the prediction of NASH in patients with NAFLD.

Alanine aminotransferase and GGT are components of the liver function index. Our study showed that serum ALT at a cut-off of 75 IU/L and GGT at a cut-off of 55 IU/L have SEs of 82.1% and 85.7%, SPs of 74.8% and 74.1%, and AUCs of 0.829 (95% CI, 0.737–0.921) and 0.892 (95% CI, 0.817–0.967), respectively, for predicting NASH in patients with NAFLD. Miyake et al. (23) reported that serum ALT could be used as a surrogate marker for the presence of NAFLD. A cross-sectional study found that individuals with ALT levels of >40 IU/L were at an increased risk of fatty liver compared with those with ALT levels of <40 IU/L (24). Another study (25) showed that serum ALT was a risk factor for NASH, which was diagnosed in 59% and 74% of the patients with normal and increased ALT, respectively (p=0.01). GGT activity is a sensitive marker of liver dysfunction (26). Tahan et al. (27) reported that an increased GGT level is a risk factor for advanced fibrosis in NAFLD. At a cut-off value of 96.5 IU/L, the AUROC curve for GGT predicting advanced fibrosis was 0.74, with an SE of 83% and an SP of 69%. Compared with our study, the study by Tahan et al. had a higher cut-off value of GGT. It is likely that there were more patients with advanced fibrosis in their study. In addition, GGT was used to predict advanced fibrosis in NAFLD in the study by Tahan et al. (27), whereas not all patients with NASH has advanced fibrosis in our study. Our study showed that, as individual parameters, ALT and GGT were more accurate as predictors of NASH, with higher SP and SE as compared with CRP and ApoB/ApoA1 ratio.

With regard to the combination of the four parameters (ALT, GGT, CRP, and ApoB/ApoA1 ratio), our study demonstrated that the combined clinical score appeared to be more accurate for the diagnosis of NASH compared with the diagnosis of NASH by using each parameter alone. The clinical score was associated with a higher AUC (0.904) compared with that associated with the use of each biomarker alone (0.829, 0.892, 0.708, and 0.712, respectively), with an SE of 90.2% and an SP of 87.0% at a cut-off of 3.8 points. Several studies investigating the use of noninvasive biomarkers or a combination of clinical and serum biomarkers for predicting NASH have been reported. Younossi et al. (28) established a combination set based on the following four parameters for the diagnosis of NASH in obese patients: CK18 fragments, M30 and M65, adiponectin level, and resistin level (the NASH Diagnostics Test), which had an SE of 95.45%, SP of 70.21%, and AUC of 0.908. Compared with our study, this study used several parameters that are not extensively applied or not convenient to measure in clinical practice, but had a lower SP (87.0% vs. 70.21%), whereas the parameters used in our study are simple and are routinely obtained in clinical settings. Poynard et al. (29) developed a model for NASH prediction (the NASH Test) using a combination of 13 variables, including age, gender, height, weight, and serum levels of TG, TC, alpha2 macroglobulin, ApoA, haptoglobin, GGT, ALT, AST, and TBIL (30), validated by Mathurin et al. for patients with morbid obesity, thereby achieving an SE of 33% and an SP of 94% for predicting patients with NASH. Compared with our study, the NASH Test for NASH prediction included more parameters, whereas our study used fewer parameters; our score also had a higher SE (90.2% vs. 33.0%). Moreover, the parameters used in our study are convenient to measure; thus, the diagnosis was simpler, easier, and inexpensive. An ideal noninvasive test for predicting NASH should not only have high SE and SP but should also be simple, inexpensive, and include parameters that are routinely measured in clinical practice.

Despite our effort, the present study had several limitations. First, as a retrospective cross-sectional study, the sample size was fairly small because of an insufficient number of patients with histologically proven NAFLD. Second, our study did not include a control group because it would have been unethical for healthy individuals to undergo a liver biopsy and medical examination in a hospital. Third, the results of our study have not been validated in additional patients with histologically proven NAFLD because no additional patients who fulfilled the inclusion criteria were available at the time; however, validation of the results will be performed in the future.

In conclusion, we constructed a clinical score for predicting NASH in patients with NAFLD in the present study. The score was based on the weighted sum of the following variables: ALT of ≥75 IU/L,

GGT of ≥55 IU/L, CRP of ≥5.6 mg/L, and ApoB/ApoA1 ratio of ≥0.8. The clinical score resulted in an AUC of 0.904 with an SE of 90.2% and an SP of 87.0% at a cut-off of 3.8 points. It must be noted that this clinical score cannot replace liver biopsy to be used as a noninvasive strategy for diagnosing NASH at present before further testing and validation. Despite this, as a predictive model that incorporates the independent risk factors indicated by the measurements of routine parameters, the clinical score may be helpful to identify NASH among patients with NAFLD and to refer patients at risk of NASH to undergo a liver biopsy for a definitive diagnosis of NASH.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Jiangsu University School of Medicine and conformed to the National Institute of Health guidelines on the clinical studies.

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author contributions: Concept - L.C., Y.X.; Design - L.C., S.J.; Supervision - Z.H., Y.X.; Resource - S.J., H.X.; Materials - Y.L., Q.C.; Data Collection &/or Processing - H.X., Q.C.; Analysis &/or Interpretation - L.C., Y.X.; Literature Search - Y.L., H.X.; Writing - L.C.; Critical Reviews - Z.H., Q.C.

Acknowledgements: We thank all our colleagues at Research and Therapy Center for Liver Disease of our hospital who cared for the patients included in this article. We also thank Professor Zhang of Jiangsu University for his help in statistic analysis in our study. The authors would especially like to thank Madam Liu of Jiangsu University School of Foreign Languages for her help in English language reviewing and revising of this article.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study has received no financial support.

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