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A simpler diagnostic formula for screening nonalcoholic fatty liver disease

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ABSTRACT

Objective: To increase the accuracy of non-invasive diagnosis of nonalcoholic fatty liver disease (NAFLD), clinical and laboratory NAFLD indicators were integrated into a diagnostic formula.

Methods: A total of 141 patients with clinically diagnosed NAFLD and 30 healthy controls were enrolled. We collected case history, body weight, height and mass index (BMI), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transpeptidase, blood urea nitrogen and blood uric acid (UA), serum creatinine, plasma total cholesterol, triglyceride, low density lipoprotein, glycosylated hemoglobin, fasting plasma glucose, fasting insulin, ultrasonic tests, Fibroscans, and other data. Linear correlation, multiple linear regressions, and receiver operating characteristic (ROC) curve methods were used to process and analyze the collected data. The performance of Fibroscan and our diagnostic formula was compared in reference to the findings of liver biopsy.

Results: The identified NAFLD diagnostic indices consisted of BMI, ALT, AST and UA. A regression formula was proposed as: CAP = 113.163 + 0.252 * ALT + 6.316 * BMI. Diagnosis of the area under the ROC curve was 0.927, the sensitivity was 87.68%, and specificity was 90%. The cutoff was 277.67 ($p < 0.01$). The accuracy of the NAFLD diagnosis with the proposed formula was significantly higher than FibroScan (82.6% vs 69.6%; $p = 0.005$).

Conclusions: NAFLD diagnosis with the proposed formula demonstrated both high sensitivity and specificity, and its accuracy was significantly higher than FibroScan. This formula only utilized non-invasive clinical and laboratory findings and the calculation was simple. It can be conveniently used for clinical diagnosis of NAFLD.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) has become increasingly prevalent and emerged as a major public health problem that affects about one billion people worldwide [1]. Pathologically, NAFLD is characterized by fatty degeneration of hepatocytes that appear diffuse in the liver [2]. NAFLD is a progressive liver disease and fatty degeneration can become nonalcoholic steatohepatitis (NASH), advance to cirrhosis, and even to liver cancer [3,4]. Since the pathogenesis of NAFLD remains poorly understood, there has been a lack of both accurate diagnostic methods, effective treatment strategies, and pharmacological treatments for NAFLD [5].

FibroScan (vibration-controlled transient elastography) is a novel, noninvasive technique to assess hepatic fibrosis and steatosis and has been evaluated in patients with chronic hepatitis B and C, and NAFLD [6]. Vibration-controlled transient elastography works by measuring shear wave velocity, which is delivered using a handheld probe (M or XL) placed in the intercostal space over the right lobe of the liver.

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FibroScan utilizes the proprietary algorithms based on the ultrasonic attenuation coefficient of vibration-controlled transient elastography, and the returning shear wave velocities are used to generate the liver stiffness measurement (LSM) and the controlled attenuation parameter (CAP), which correlate with hepatic fibrosis and steatosis, respectively [7].

Liver biopsy based histology is the only reliable and sensitive diagnosis of NAFLD, hepatic steatosis, NASH, and liver fibrosis since those pathologic changes tend to be disseminated throughout liver. However, it cannot be routinely employed as patients often decline a liver biopsy procedure because of its invasive nature and risk for bleeding. In addition, biopsied liver tissues may not always be representative if the underlying liver injury is not evenly distributed among the whole liver [8]. Also, imaging examinations of the liver are not affordable for all patients. Therefore, there is a need to develop a simple, but accurate, non-invasive NAFLD diagnosis. Several investigations have been focused on identifying serum biomarkers of NAFLD, and a few studies [9–11] have used Fibroscan as a reference, aimed at establishing noninvasive diagnostic criteria. The purpose of this study was to develop a noninvasive diagnostic formula consisting of routine clinical and laboratory indicators and evaluate and compare the accuracy of this formula-based NAFLD diagnosis to FibroScan using histology as the definitive diagnostic reference.

2. Materials and methods

2.1. Participants

A total of 171 participants were enrolled and divided into a NAFLD group ($n = 141$ patients) and a non-NAFLD control group ($n = 30$ cases). NAFLD diagnosis followed the criteria outlined by the guidelines of Asia-Pacific Working Party on Non-alcoholic Fatty Liver Disease [12] and used both FibroScan and laboratory findings. The upper limit of the controlled attenuation parameter (CAP) of FibroScan for a normal liver was set as 238db/m [7] by average published values. Patients were excluded if they had autoimmune liver disease, drug-induced liver injury, alcoholic fatty liver disease, and viral liver disease. Written informed consent was obtained from all participants. This study was approved by the ethics committee of the First Affiliated Hospital of Xi'an Medical University and The First Affiliated Hospital of Wenzhou Medical University.

2.2. Clinical and laboratory findings

Demographic, clinical, and laboratory characteristics of each participant were collected, including gender, age, height, weight, BMI, medical history, ALT, AST, GGT, blood urea nitrogen (BUN), UA, serum creatinine (CR), plasma total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL), low density lipoprotein (LDL), glycosylated hemoglobin (HBA1c), fasting plasma glucose (FPG), fasting insulin (FINS), platelet (PLT), and ultrasound and Fibroscan examinations.

2.3. Fibroscan scanning

FibroScan-502 (Echosens, France) was used to quantify hepatic steatosis. The CAP value was expressed as dB/m. FibroScan was operated by two doctors who have obtained the FibroScan® operator certificate. The detection points were selected between the right axillary front and the median axillary line (7, 8 or 8, 9 intercostal Spaces). The measurement consisted of 10 continuous effective detections, the median value was computed as the reported value. The operators of the FibroScan were blinded to the clinical and biochemical data of the patients. As suggested by Lédinghen and colleagues, CAP values can classify fatty liver into 4 levels (S0, S1, S2, and S3). S0 or normal liver with CAP < 238 db/m, an indicator < 10% liver fat; S1 or mild fatty liver (11–33% fatty liver) with 238 db/m < CAP < 259 db/m; S2 or moderate fatty liver (34%~66%) with 259 db/m < CAP < 292 db/m; S3 or severe fatty liver (> 67%) with CAP > 292 db/m [13].

2.4. Liver biopsy

In this study, 92 cases underwent liver biopsy, and the detailed histology findings for each biopsy were provided by the First Affiliated Hospital of Wenzhou Medical University. The NAFLD activity score (NAS) system was used to differentiate NAFLD from non NAFLD. The NAS consists of 0 to 8 points that represent the summation of the degree of steatosis (0–3), lobular inflammation (0–3), and hepatocellular ballooning (0–2). Histology was scored by an experienced liver pathologist who was blinded to the clinical data, treatment allocation, and imaging findings.

2.5. Statistical analyses

The statistical methods included correlation analysis, linear regression and ROC curve. A diagnosis formula was developed using clinical and laboratory findings. The statistical description is expressed by $\overline{X}\pm S$, upon a normal distribution of the measurement data. Student's *t*-test was used to computer differences among groups. Linear correlation was used to determine correlation, and regression equation was constructed by multivariate linear regression model. $p < 0.05$ was considered statistically significant.

2.6. Data availability

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

2.7. Study/ethics approval

This study was approved by the ethics committee of the First Affiliated Hospital of Xi'an Medical University and The First Affiliated Hospital of Wenzhou Medical University. All experiments were performed in accordance with relevant guidelines and regulations.

3. Results

3.1. Baseline characteristics of the enrolled participants

There were no significant differences in age and sex between NAFLD and non-NAFLD groups ($p > 0.05$). Comparisons of laboratory findings are listed in Table 1.

3.2. Indicators related to fat content

CAP values of Fibroscan are viewed as an indirect indicator for liver fat content. We used linear correlation analysis to investigate the relationship between BMI, ALT, AST, GGT, BUN, UA, CR, TC, TG, HDL, LDL, HBA1c, FPG, FINS, PLT, and CAP. A number of factors were found to correlate with the CAP, as arranged in descending order: BMI ($r = 0.436$, $p < 0.01$), ALT ($r = 0.356$,$p < 0.01$), AST ($r = 0.317$,$p < 0.01$), UA ($r = 0.250$,$p < 0.01$) (Fig. 1, 2, 3, and 4). Within a certain range, the higher value of the correlated indicators, the greater the fat content.

3.3. Construction of regression formula

The uncovered correlations between CAP and BMI, ALT, AST and UA, prompted us to integrate each factor one by one to determine if a combination of them would yield a comprehensive formula for diagnosis. Through regression analysis, we found that the regression coefficients of AST, GGT, FFA and UA were not statistically significant.

Table 1
Baseline laboratory findings between NAFLD and non-NAFLD group.

observation index	NAFLD	Non-NAFLD	*t* value	*p* value
	$\bar{x} \pm s$	$\bar{x} \pm s$		
ALT (U/L)	76.462 ± 61.271	37.033 ± 19.18	−6.292	0.000
AST (U/L)	51.173 ± 28.833	28.833 ± 9.656	−6.433	0.000
GGT (U/L)	73.555 ± 116.340	41.367 ± 30.262	−1.503	0.135
BUN (mmol/L)	4.678 ± 1.298	4.503 ± 1.112	−0.673	0.502
UA (μmol/L)	416.759 ± 116.447	357.724 ± 86.862	−3.106	0.003
CR (μmol/L)	68.977 ± 14.788	73.003 ± 10.558	1.390	0.166
TC (mmol/L)	8.058 ± 37.146	4.938 ± 1.416	−0.451	0.652
TG (mmol/L)	2.529 ± 1.604	1.701 ± 0.784	−4.517	0.000
HDL (mmol/L)	1.064 ± 0.248	1.071 ± 0.187	0.136	0.892
LDL (mmol/L)	7.610 ± 39.507	3.096 ± 1.049	−0.614	0.540
HBAIC (%)	6.043 ± 1.361	5.557 ± 0.811	−1.660	0.099
FPG (mmol/L)	5.807 ± 1.937	5.449 ± 1.853	−0.925	0.356
FINS (mIU/L)	10.275 ± 5.882	6.692 ± 3.103	−0.694	0.488
PLT (10^9/L)	222.907 ± 67.818	222.577 ± 64.934	−0.426	0.982
BMI (kg/m^2)	27.119 ± 2.764	22.911 ± 2.065	−7.870	0.000

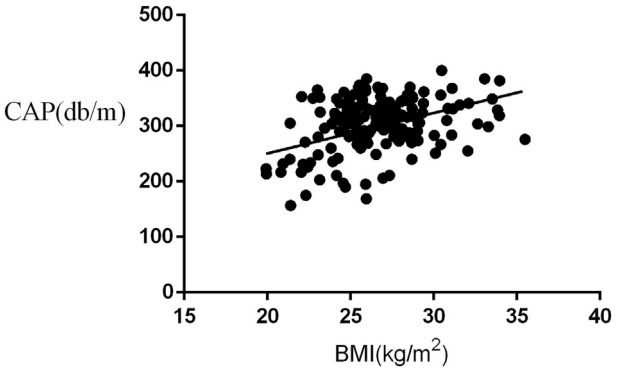

Fig. 1. The correlation between BMI and CAP. We used linear correlation analysis to investigate the relationship between BMI and CAP (r = 0.436, $p < .01$).

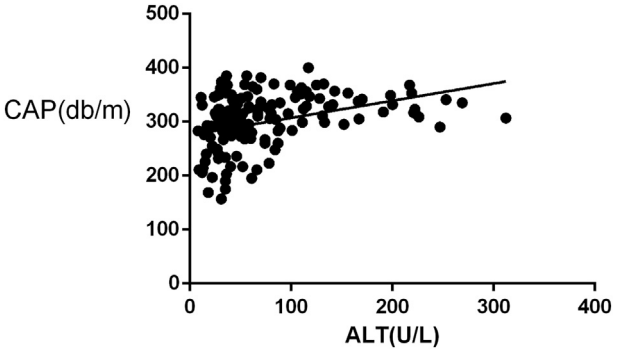

Fig. 2. The correlation between ALT and CAP. We used linear correlation analysis to investigate the relationship between ALT. And CAP (r = 0.356, $p < .01$).

Finally, the regression equation between the three groups of CAP, ALT and BMI was constructed. we found that an inclusion of CAP, ALT, and BMI into the regression formula resulted in a better diagnostic performance. The multiple regression analysis showed statistical significance when the regression model of CAP was tested (F = 30.117, $p < 0.01$). When the correction coefficient in the formula was $R^2 = 0.258$, the ratio of variance induced by the regression in the total variation was 25.8%. The most important index for CAP was BMI (β = 0.377, $p = 0.001$) and ALT (β = 0.281, $p = 0.012$). The regression formula of CAP is expressed as: CAP = 113.163 + 0.252 * ALT + 6.316 * BMI (Table 2).

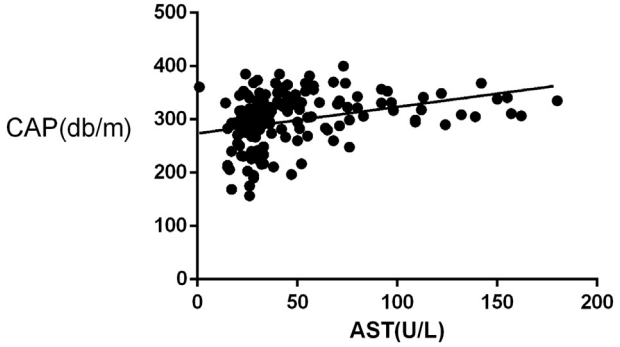

Fig. 3. The correlation between AST and CAP. We used linear correlation analysis to investigate the relationship between AST and CAP (r = 0.317, $p < .01$).

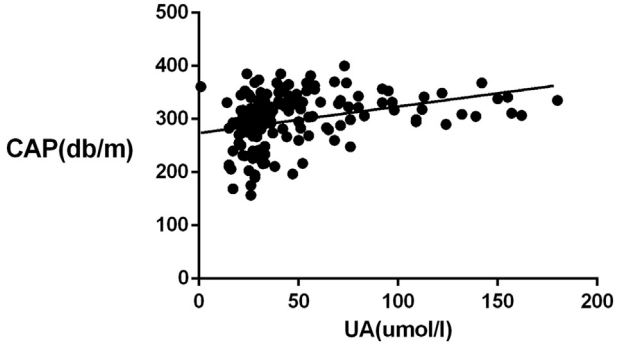

Fig. 4. The correlation between UA and CAP. We used linear correlation analysis to investigate the relationship between UA and CAP (r = 0.250, $p < .01$).

Table 2
Regression analysis of factors that affected the CAP value index.

Independent variable	B	S.E.	B	T	Adjust R^2	F
Constant	113.163	29.723		3.807	0.258	30.107***
ALT	0.252	0.061	0.281	4.122*		
BMI	6.316	1.144	0.377	5.522**		

*$p < .05$. **$p < .05$, ***$p < .05$.

3.4. The establishment of ROC curve

According to Lédinghen et al. [13], CAP = 238 was a cutoff value that separated normal liver fat from a fatty liver. We verified this value calculated by the constructed regression formula and generated the ROC curve (Fig. 5): the area under ROC curve was 0.927, sensitivity and specificity were 87.68% and 90.00%, respectively and the cut-off value was 277.67 ($p < 0.01$) for diagnosis of fatty liver (Table 3).

3.5. Comparison of the performance by FibroScan and diagnostic formula

The histological findings from liver biopsy, which were graded using the NAS scoring system, were used as a reference for evaluating the performance of FibroScan and the diagnostic formula generated by this study. The critical value of FibroScan remained set at 238db/m, while it was 277.67 with our diagnostic formula. Among 92 patients with liver biopsies, the diagnostic consistency was 82.6% between histology and the formula, significantly higher than 69.6% between histology and FibroScan ($\chi^2 = 7.796$, $p = 0.005$, Table 4). To understand why a quarter of the patients showed inconsistencies among the three methods, we took a more careful look at the case histories of these patients. Our analysis showed that most of them had diabetes or hypertension. The diagnosis performance of our formula was improved by

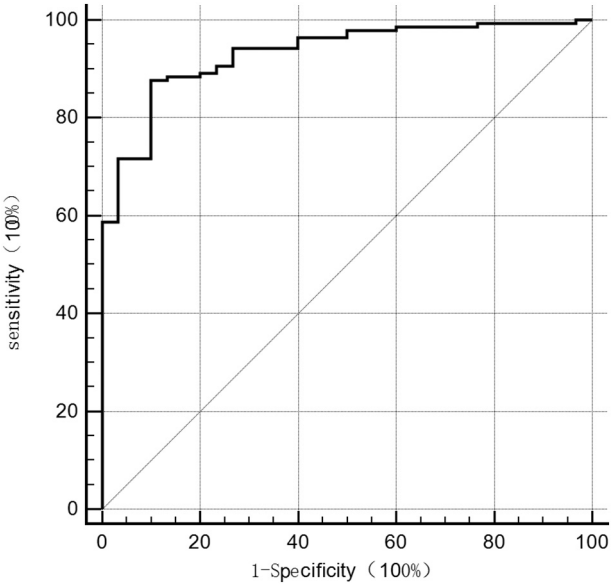

Fig. 5. The ROC curve generated by the diagnostic formula. We verified this value calculated by the constructed regression formula and generated the ROC curve: the area under ROC curve was 0.927, sensitivity and specificity were 87.68% and 90.00%, respectively and the cut-off value was 277.67 ($p < .01$) for diagnosis of fatty liver.

Table 3
Sensitivity, specificity, and area description of the curve in the diagnosis formula.

Equation name	Sensitivity	Specificity	Area under curve	Cut-off	*p* value
CAP	87.68%	90.00%	92.7%	277.67	< 0.0001

Table 4
Performance in diagnosis between the formula and FibroScan

Diagnostic methods	Performance		χ^2	*p* value
	True	False		
Diagnostic formula	76 (82.6%)	16 (17.4%)	7.796	0.005
Fibroscan	64 (69.6%)	28 (30.4%)		

excluding those patients from the analysis. Thus, the diagnostic formula performed much better if the subjects did not have concurrent diabetes or hypertension.

3.6. Clinical utility for screening fatty liver in subjects without risk factors

It has been suggested that patients with metabolic diseases should undergo screening fatty liver. The guidelines for the diagnosis and treatment of non-alcoholic fatty liver disease in China (2010 Revised Edition) also highlights a number of metabolic risk factors for fatty liver. Patients with visceral obesity, type 2 diabetes, dyslipidemia, hypertension, metabolic syndrome, and recent gain or loss in body weight are required to assess liver function with upper abdominal ultrasonography, in addition to assessing the nondestructive injuries of the heart, brain and kidney [14]. However, those patients who do not have metabolic risk factors, should be subject to evaluation with the diagnostic formula. If the critical value is greater than the cutoff, it may suggest a fatty liver. The screening performance with the formula was satisfactory when tested in this cohort. A proposed algorithm for screening fatty liver is shown in Fig. 6.

3.7. Verification of diagnostic equation in different populations

In order to further verify the accuracy of the noninvasive diagnosis model, 68 more patients with liver histology were included. In this extended study, we compared the accuracy between this diagnostic model and Fibroscan using the liver histology as reference. The results showed that the diagnostic consistency was 88.2% between histology and the formula, significantly higher than 67.6% between histology and FibroScan ($\chi^2 = 8.382$, $p = 0.004$, Table 5), and the diagnostic model remained superior to Fibroscan.

4. Discussion

Several modalities including Fibroscan and imaging of the liver can be used as noninvasive tools for diagnosis of fatty liver. Both imaging techniques and Fibroscan demonstrate the limitations of diagnosis. For instance, when fat content in the liver is below 30%, the abdominal ultrasound shows poor sensitivity. FibroScan has become increasingly popular, but it has only moderate accuracy in diagnosing patients with significant fibrosis (fibrosis stage 2 or greater) [14]. On the other hand, several makers may be used to reflect lipid metabolism and the associated liver injury. The objective of our study was to propose and test an objective and practical NAFLD diagnostic model by combining common markers that indicate abnormal lipid metabolism and liver injury. After multi-run tests, we found a formula that consisted of BMI and ALT values could provide better diagnosis of fatty liver than FibroScan.

There are three types of established formulas for diagnosis of NAFLD: The first one was the fatty liver index (FLI) [15], with 61% sensitivity and 86% specificity, and 85% area under the ROC curve. The second one was the NAFLD liver fat score [16], which had 86% sensitivity, 71% specificity and 86% area under the ROC curve. The third one was SteatoTest [17], delivering 38% sensitivity, 81% specificity, and 80% area under the ROC curve. In this study, through regression analysis, we first evaluated the correlation of individual biochemical markers with fatty liver and found that CAP, ALT, AST, BMI, GGT and UA correlated with fatty liver relatively well. Then through combinational tests, we generated a new diagnostic formula of CAP = 113.163 + 0.252 * ALT + 6.316 * BMI. This formula showed 87.68% sensitivity, 90.00% specificity and 92.7% area under the curve of ROC, which were significantly higher than the performance by any of the three cited formulas above.

The number of markers required for our formula was just two, the fewest comparing the three established ones. It is easy to use in clinic. This formula can be used for screening patients if fatty liver or NAFLD is suspected. Also, we proposed an algorithm that guides physicians to use this formula and other tools for diagnosis and management of patients with fatty liver.

Insulin resistance is commonly associated with NAFLD as well as metabolic diseases, many of which can be secondary to NAFLD. The risk for type 2 diabetes mellitus is increased by 1.86 times (95% CI: 1.76–1.95), for metabolic syndrome (MetS) increased by 3.22 times (95% CI: 3.05–3.41) and cardiovascular events increased by 1.64 times (95% CI: 1.26–2.13) after 5 to 10 years of follow-up in NAFLD patients [18,19]. Even if other or traditional MetS and cardiovascular risk factors were effectively controlled, the incidence of coronary heart disease in NAFLD patients was significantly increased [20]. Therefore, it is of great importance to diagnose NAFLD early and mitigate other metabolic risk factors. We used to recommend screening NAFLD among population with metabolic risk factors, but overlooked non-metabolic risk factors. Under our diagnostic model, NAFLD patients with non-metabolic risk factors can be effectively screened to establish early diagnosis in addition to the recognition of NAFLD patients with metabolic risk factors.

There were a few limitations in this study. First, the size of our sample included in this study was relatively small, and Our next step is to expand the sample size to incorporate different regions and ethnic

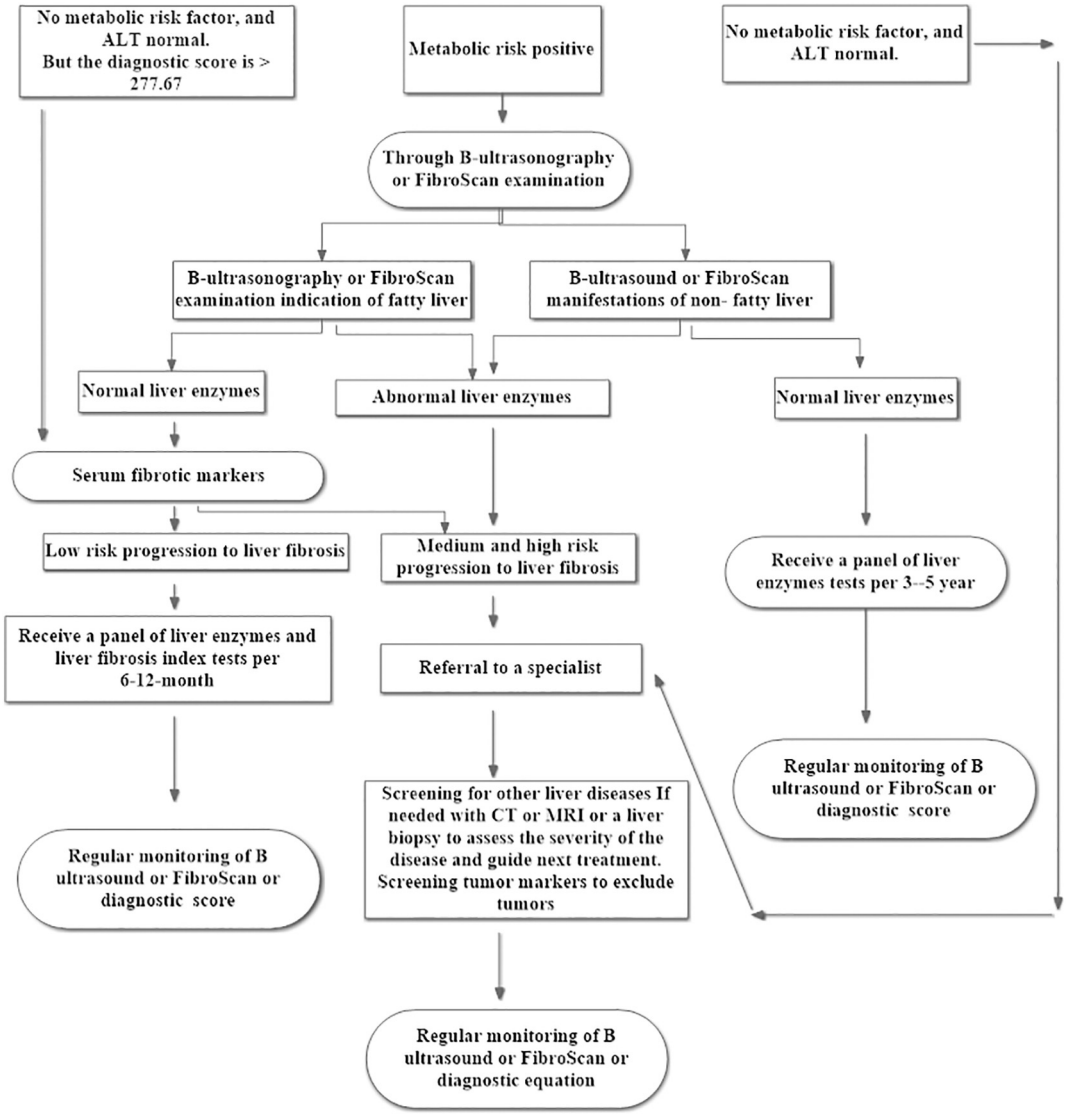

Fig. 6. A proposed algorithm for screening fatty liver. Those patients who do not have metabolic risk factors, should be subject to evaluation with the diagnostic formula. If the critical value is greater than the cutoff, it may suggest a fatty liver. The screening performance with the formula was satisfactory when tested in this cohort.

Table 5
Performance in diagnosis between the formula and FibroScan

Diagnostic methods	Performance		χ^2	*p* value
	True	False		
Diagnostic formula	60 (88.2%)	8 (11.8%)	8.382	0.004
Fibroscan	46 (67.6%)	22 (32.4%)		

groups into our research. The reliability of the diagnostic equation will be further enhanced through multicenter, large cohort studies. Second, this formula is not suitable for patients with severe liver injury or high ALT level; Third, a consensus on the critical value of Fibroscan CAP in the diagnosis of fatty liver remains to be established. Thus, a change in the consensus CAP value may prompt revisions of our formula.

In conclusion, we used the histological scores of fatty livers as reference to the diagnosis of NAFLD and non-NAFLD patients, developed a simpler formula that only utilizes BMI and ALT value for screening patients with fatty liver. The performance of this new formula was better than Fibroscan, in addition to the superiority to the three published formulas. We proposed an algorithm to guide the use of this formula and other tools for diagnosis and management of NAFLD patients.

Author contributions

Conception and design: Gong Feng, Man Mi, and Ming-Hua Zheng. Experiments performance: Gong Feng, Na He, Yi-Fan Zhou, and Ya-ming Li. Formal analysis: Xue-Ping Li, Chunyan Niu, Ke-lin Zhang, and Ya Li. Supervision: Man Mi, Xue-Ping Li, and Chunyan Niu. Article drafting: Gong Feng and Na He. All authors reviewed the manuscript.

Declaration of conflicting interests

The author(s) declared no conflicts of interest.

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