Journal of Clinical and Translational Hepatology **2021** vol. 9(5) | 607–614
DOI: 10.14218/JCTH.2021.00058

Original Article

Metabolic Disorders Combined with Noninvasive Tests to Screen Advanced Fibrosis in Nonalcoholic Fatty Liver Disease

Yi-Wen Shi[1#], Fang-Ping He[2#], Jin-Jun Chen[3], Hong Deng[4], Jun-Ping Shi[5], Cai-Yan Zhao[6], Yu-Qiang Mi[7], Zheng-Sheng Zou[8], Yong-Jian Zhou[9], Fu-Sheng Di[10], Rui-Dan Zheng[11], Qin Du[12], Jia Shang[13], Rui-Xu Yang[1], Branko Popovic[14], Bi-Hui Zhong[15*] and Jian-Gao Fan[1*]

[1]Center for Fatty Liver, Department of Gastroenterology, Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai Key Lab of Pediatric Gastroenterology and Nutrition, Shanghai, China; [2]Department of Gastroenterology II, The First Affiliated Hospital of Xinjiang Medical University, Xinjiang Uygur Autonomous Regions, Ürümqi, China; [3]Hepatology Unit, Department of Infectious Diseases, Nanfang Hospital, Southern Medical University, Guangzhou, China; [4]Department of Infectious Diseases, the Third Affiliated Hospital, Sun Yat-Sen University, Guangzhou, China; [5]The Affiliated Hospital of Hangzhou Normal University, Hangzhou, China; [6]Department of Infectious Disease, The Third Hospital of Hebei Medical University, Shijiazhuang, China; [7]Department of Infectious Diseases, Tianjin Second People's Hospital, Tianjin, China; [8]Department of Liver Disease, Chinese PLA General Hospital, the Fifth Medical Center of Chinese PLA General Hospital, Beijing, China; [9]Department of Gastroenterology and Hepatology, Guangzhou First People's Hospital, School of Medicine, South China University of Technology, Guangzhou, China; [10]Department of Endocrinology and Metabolism, The Third Central Hospital of Tianjin, Tianjin, China; [11]Diagnosis and Treatment Center for Liver Diseases, Zhengxing Hospital, Zhangzhou, China; [12]Department of Gastroenterology, the Second Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, China; [13]Department of Infectious Diseases, Henan Provincial Peoples' Hospital Zhengzhou Zhengzhou, China; [14]Sanofi-Aventis Deutschland GmbH, Frankfurt, Germany; [15]Department of Gastroenterology, The First Affiliated Hospital, Sun Yat-Sen University, Guangzhou, China

Received: 6 February 2021 | **Revised:** 30 March 2021 | **Accepted:** 5 April 2021 | **Published:** 23 April 2021

Abstract

Background and Aims: Nonalcoholic fatty liver disease (NAFLD) is associated with metabolic disorders. This study aimed to explore the role of metabolic disorders in screening advanced fibrosis in NAFLD patients. ***Methods:*** A total of 246 histologically-proven NAFLD patients were enrolled across 14 centers. We compared the severity of fibrosis in patients with different components of metabolic disorders. Based on standard noninvasive tests and metabolic disorders, we developed new algorithms to identify advanced fibrosis. ***Results:*** Metabolic syndrome (MetS) was frequent in NAFLD patients (133/246, 54%). Patients with MetS had a higher proportion of significant fibrosis (p=0.014) and higher LSM values (9.2 kPa, vs. 7.4 kPa, p=0.002) than those without MetS. Patients with more metabolic disorders had higher fibrosis stages (p=0.017). Reduced high-density lipoprotein cholesterol (odds ratio [OR]: 2.241, 95% confidence interval [CI]: 1.004–5.002, p=0.049) and raised fasting glucose (OR: 4.500, 95% CI: 2.083–9.725, p<0.001) were significantly associated with advanced fibrosis. Using these two metabolic disorders as a screening tool, a sensitivity, specificity and accuracy of 92%, 81% and 83% was achieved, respectively. With the new algorithms combining metabolic disorders with noninvasive measurements, the number of patients requiring liver biopsy was reduced, especially in combination with the Fibrosis-4 score and metabolic disorders (36% to 17%, p<0.001). In addition, this stepwise algorithm could achieve a high accuracy (85%) and high negative predictive value (93%). ***Conclusions:*** Metabolic disorders should be taken into consideration in the diagnosis of advanced fibrosis. With further validation and investigation, new algorithms could be recommended in primary care units to spare patients from unnecessary referral and liver biopsies.

Citation of this article: Shi YW, He FP, Chen JJ, Deng H, Shi JP, Zhao CY, *et al*. Metabolic disorders combined with noninvasive tests to screen advanced fibrosis in nonalcoholic fatty liver disease. J Clin Transl Hepatol 2021;9(5):607–614. doi: 10.14218/JCTH.2021.00058.

Keywords: Nonalcoholic fatty liver disease; Liver fibrosis; Metabolic syndrome; Noninvasive measurement.

Abbreviations: APRI, aspartate aminotransferase to platelet ratio index; AUROC, Area under the ROC curve; BMI, body mass index; CI, confidence interval; FIB-4, fibrosis-4 score; FPG, fasting plasma glucose; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment-insulin resistance; LSM, liver stiffness measurement; MAFLD, metabolic dysfunction-associated fatty liver disease; MetS, metabolic syndrome; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NFS, NAFLD fibrosis score; ORs, odds ratios; SAF, steatosis, activity score and fibrosis stages; T2DM, type 2 diabetes mellitus; TG, triglyceride.

[#]Contributed equally to this work.

[*]**Correspondence to:** Bi-Hui Zhong, Department of Gastroenterology, The First Affiliated Hospital, Sun Yat-Sen University, No. 58 Zhongshan Road II, Yuexiu District, Guangzhou, Guangdong 510080, China. ORCID: https://orcid.org/0000-0002-3089-8152. Tel: +86-20-8775-5766-8172, Fax: +86-20-8733-2916, E-mail: Sophiazhong@hotmail.com; Jian-Gao Fan, Center for Fatty Liver, Department of Gastroenterology, XinHua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai Key Lab of Pediatric Gastroenterology and Nutrition, Shanghai 200092, China. ORCID: https://orcid.org/0000-0002-8618-6402. Tel/Fax: +86-21-2507-7340, E-mail: fanjiangao@xinhuamed.com.cn

Introduction

Nonalcoholic fatty liver disease (NAFLD) is closely associated with the features of metabolic syndrome (MetS), including insulin resistance, hyperglycemia and dyslipidemia. Since the global epidemic of obesity has increased metabolic dysfunction, the health burden of NAFLD is becoming enormous.[1] A study from the Third National Health and Nutrition Examination Survey database reported that 95% of NAFLD patients had type 2 diabetes mellitus (T2DM), obesity or other metabolic abnormalities.[2] Approximately 43% of patients with MetS had NAFLD.[3] There were also opinions that NAFLD was a representation of MetS in the liver.

It has been reported that overweight/obese NAFLD patients have more severe histological features, including higher fibrosis scores, than lean patients.[4] Among these, metabolically unhealthy obese patients had a significantly higher prevalence of advanced liver fibrosis (F3–F4) than metabolically healthy obese ones.[5] Even in nonobese patients with NAFLD, metabolic-related diseases were also common. Nonobese NAFLD patients had impaired glucose tolerance, low adiponectin concentrations and a distinct metabolite profile compared with patients without steatosis.[6] NAFLD has a universal association with insulin resistance, which plays an essential role in the development of steatohepatitis and fibrosis.

Individuals with raised fasting glucose or T2DM and other metabolic abnormalities of MetS have an increased risk of advanced fibrosis.[3] We know that advanced fibrosis is directly associated with liver-related events.[7] Therefore, metabolic abnormalities could predict a worse long-term prognosis in NAFLD patients. Hence, metabolic disorders should be considered in screening fibrosis. Currently, the application of noninvasive diagnostic measurements in clinical practice is still insufficient. The diagnostic performance, accessibility and cost-effectiveness all need to be improved. Thus, this study aimed to explore the role of metabolic disorders in screening for liver fibrosis.

Methods

Study design and population

This was an observational, multicenter, cross-sectional registry study that enrolled patients with liver biopsy-proven NAFLD in 14 participating sites across mainland China from July 4, 2016 to August 9, 2018. The procedures of this study were in accordance with the ethical standards of the responsible committee on human experimentation and conformed to the ethical guidelines of the latest version of the Declaration of Helsinki. This retrospective study did not involve any sensitive patient data, so informed consent was not required. The protocol of the study was registered at http://www.chictr.org.cn (ChiCTR-OOC-16007902).

Patients were included if they (a) were aged 18–65 years, (b) had received liver biopsy within 6 months before enrollment and biopsy sample could be collected for re-evaluation, and (c) had ≥5% hepatic steatosis on liver biopsy and were diagnosed with NAFLD. Patients were excluded if they (a) had other chronic liver diseases, including viral hepatitis, alcoholic liver diseases, toxic liver damage, autoimmune liver disease, drug-induced liver injury, Wilson's disease and other genetic liver diseases, (b) had significant alcohol consumption of >140 g/week for men or >70 g/week for women within the past 12 months, (c) had end-stage liver disease, such as decompensated cirrhosis or liver cancer, or (d) were pregnant or breastfeeding.

Data collection, laboratory, imaging and liver biopsy examination

Demographic and anthropometric characteristics, medical history and metabolic disorders were collected at enrollment. Weight and height were used to calculate body mass index (BMI=weight/height^2). Medical history was recorded in detail, including comorbid diseases, alcohol consumption, smoking status and comedications. Clinical and laboratory information of the patients, including blood biochemical parameters, was obtained within 1 week before or after liver biopsy. The cardiovascular disease risk score was calculated according to the Framingham general risk score algorithm (2008). The controlled attenuation parameter and liver stiffness measurement (LSM) were performed within 1 week of biopsy using the FibroScan 502 instrument (Echosens, Paris, France).

Liver biopsy samples of eligible patients were collected for histopathological rereading. The biopsy specimens were stained with hematoxylin and eosin, reticulin, and Masson's trichrome. Pathologists at each site read biopsy slices in terms of steatosis, activity score and fibrosis stages (SAF) and provided a standard report according to the Fatty Liver Inhibition of Progression (commonly known as FLIP) Algorithm.[8] The diagnosis of NAFLD was based on the EASL-EASD-EASO Clinical Practice Guidelines for the management of NAFLD (2016).[9] Nonalcoholic steatohepatitis (NASH) was defined as the presence of steatosis with inflammation and ballooning. Significant fibrosis was defined as fibrosis stage ≥F2, while advanced fibrosis was defined as stage F3 and F4.[8] Liver biopsies were also used to differentiate other liver diseases in patients. The study protocol was published previously.[10] Qualified researchers may request access to patient-level data and related study documents.

Definitions of metabolic disorders and noninvasive fibrosis tests

MetS consisted of central obesity plus any two of the following metabolic disorders: elevated triglyceride (TG), reduced high-density lipoprotein cholesterol (HDL-C), elevated blood pressure and raised fasting glucose, according to the guideline from the International Diabetes Foundation (2005).[11] Central obesity was defined as increased waist circumference, with thresholds of ≥90 cm in men and ≥80 cm in women. Elevated TG was defined as fasting TG ≥150 mg/dL or being on TG therapy. Reduced HDL-C was defined as <40 mg/dL in men and <50 mg/dL in women or being on HDL-C therapy. Elevated blood pressure was defined as ≥130/85 mm Hg or being on hypertension therapy. Raised fasting glucose was defined as ≥100 mg/dL or previously diagnosed T2DM. Insulin resistance was defined as homeostasis model assessment-insulin resistance (HOMA-IR) score ≥2.5, calculated as fasting insulin (mU/L) × fasting glucose (mmol/L)/22.5.

NAFLD fibrosis score (NFS) was calculated as: −1.675+0.037×age (years)+0.094×BMI (kg/m^2) +1.13×impaired fasting glycemia or diabetes (yes=1, no=0) +0.99×aspartate aminotransferase/alanine aminotransferase ratio-0.013×platelet (×10^9/L)−0.66×albumin (g/dL). The cut-off value of −1.455 was used to rule-out advanced fibrosis with 90% sensitivity, and 0.676 was used to rule-in advanced fibrosis with 90% specificity.[12] Fibrosis-4 (FIB-4) was calculated as age×aspartate aminotransferase (U/L)/platelet (×10^9/L) ×alanine aminotransferase$^{1/2}$ (U/L). Two diagnostic cut-offs, namely 1.30 and 3.25, corresponding to the 90% sensitivity and 90% specificity thresholds, were also used to diagnose advanced fibrosis.[13] We used 7.9 kPa as a cut-off

of LSM to exclude advanced fibrosis and 9.6 kPa to diagnose advanced fibrosis according to the published data.[14]

Statistical analysis

The database for final analysis was locked on December 5, 2018. Statistical analyses were performed using SPSS software (version 25.0; IBM Corp., Armonk, NY, USA). The comparison of LSM values between groups with different numbers of metabolic disorders was carried out by one-way ANOVA. Univariate and multivariate binary logistic regression analyses were applied to define risk factors for advanced fibrosis. All related factors were calculated in the multivariate model using the forward stepwise (conditional) method. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to evaluate the effect.

We used receiver operating characteristic curves (commonly known as ROCs) to assess the accuracy and to identify optimal cut-offs. The area under the ROC curve (commonly known as AUROC), diagnostic OR and diagnostic accuracy were calculated to assess the overall diagnostic performance. In the new stepwise algorithms, two cut-off values of each noninvasive assessment measurement were applied to determine advanced fibrosis. The second step was applied for the patients in the "gray zone" of the first step, and the patients in the final "gray zone" would then be recommended for a liver biopsy. Overall diagnostic indexes, including sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio and negative likelihood ratio, were calculated.

Results

Demographic and clinical characteristics of NAFLD patients

Eligible biopsy samples were obtained from 250 patients enrolled in the study. Two patients were excluded for alcohol abuse, and another two patients were excluded for insufficient steatosis of less than 5% during histological re-examination. Finally, 246 patients with histologically-proven significant steatosis were diagnosed with NAFLD and included in the final analysis. Approximately 61% (151/246) of patients had moderate or severe steatosis, and 84% (207/246) of patients had NASH. The median SAF score was 5 (interquartile range: 4, 7). A total of 76 (31%) patients had significant fibrosis, and 38 (15%) patients had advanced fibrosis.

Metabolic disorders were frequent among the patients in this study. Approximately 76% (178/234) of patients had central obesity, and more than half (133/246, 54%) of the patients met the criteria for MetS. Patients with MetS had higher BMI, higher waist circumference and higher hip circumference (all $p<0.001$). These patients also had a higher proportion of hypertension and T2DM ($p<0.001$ and $p=0.001$, respectively). It was also not surprising that patients with MetS appeared to have worse metabolic status, including fasting plasma glucose (FPG), glycosylated hemoglobin, HOMA-IR, TG and HDL-C (Table 1).

Metabolic disorders were associated with liver fibrosis

NAFLD patients with MetS showed more severe fibrosis. Patients in the MetS group had a significantly higher proportion of significant fibrosis than those without MetS (50 cases, 38% vs. 26 cases, 23%, $p=0.014$). The proportion of advanced fibrosis in patients with MetS (23/133, 17%) was also higher than that in patients without MetS (15/113, 13%) but there was no significant difference ($p=0.245$). Patients with MetS had higher overall LSM values than patients without MetS (9.2, 7.2–13.2 kPa vs. 7.4, 5.5–10.1 kPa, $p=0.002$) and similar IQR values (1.2, 0.7–2.2 vs. 0.8, 0.6–1.5, $p=0.174$).

When compared from the perspective of the numbers of metabolic disorders included within MetS (central obesity, elevated TG, reduced HDL-C, elevated blood pressure and raised FPG according to the diagnosis criteria), patients with more disorders had significantly more severe fibrosis ($p=0.017$; Fig. 1). In patients without any of the metabolic disorders, no patients had advanced fibrosis. In patients with only one metabolic disorder, there were no cirrhotic patients. In patients with five metabolic disorders, 36% had advanced fibrosis. We also compared the LSM values among patients with different numbers of metabolic disorders. NAFLD patients with more metabolic disorders had significantly higher LSM values ($p<0.001$; Fig. 1). In addition, the number of disorders showed a linear correlation with the LSM values ($p=0.005$).

Reduced HDL-C levels and raised fasting glucose were risk factors for fibrosis

To determine which of the metabolic disorders were significant risk factors for advanced fibrosis, we performed univariate analysis and multivariate analysis among the five components of MetS. Raised FPG (OR: 4.500, 95% CI: 2.083–9.725, $p<0.001$) and reduced HDL-C (OR: 2.241, 95% CI: 1.004–5.002, $p=0.049$) were the most important risk factors (Table 2). Approximately 28% (27/98) of patients with raised FPG and 19% (27/141) of patients with reduced HDL-C had advanced fibrosis.

Next, we used the two metabolic disorders as risk factors to screen advanced fibrosis. A total of 66 patients had neither of these metabolic disorders, 121 patients had either reduced HDL-C levels or raised fasting glucose, and 59 patients had both. The proportion of significant fibrosis and advanced fibrosis was significantly different among the three groups (both $p<0.001$). There was also a trend towards a higher proportion of cirrhosis in patients with more metabolic disorders (1 case vs. 2 cases vs. 3 cases, $p=0.319$). Patients with more metabolic disorders also had higher LSM values (7.3, 5.3–9.5 kPa; 9.0, 6.6–12.1 kPa; and 10.0, 7.0–14.2 kPa, respectively, $p<0.001$; Table 3).

New algorithms improved the diagnostic performance of advanced fibrosis

We then used these metabolic disorders as a screening tool for advanced fibrosis. Patients with both raised FPG plus reduced HDL-C were ruled-in to the consideration of a diagnosis of advanced fibrosis; patients with neither of these were ruled out and patients with either of these were considered in the gray zone. This new diagnostic tool (MetDis) could achieve a sensitivity, specificity and accuracy of 92%, 81% and 83%, respectively. We also evaluated the diagnostic performance of three standard noninvasive tests at their best Youden's index to identify advanced fibrosis (Supplementary Table 1). This new algorithm had significantly better diagnostic performance than LSM ($p<0.001$; Table 4).

We also combined metabolic disorders with standard noninvasive tests using their published cut-offs to form several new stepwise algorithms (Supplementary Table 2). The specificity of the new algorithms was also improved com-

Table 1. Clinical characteristics between patients with and without metabolic syndromes

Variable	Patients with MetS	Patients without MetS	*p*
Number of patients	133	113	–
Age in years	42±13	38±12	0.037
Male, %	89, 67%	88, 78%	0.057
Body mass index in kg/m^2	28.9±4.2	25.6±3.2	<0.001
Smoking, %	20,15 %	10, 9%	0.146
Alcohol intake, %	48, 36%	46, 41%	0.458
Hypertension, %	42, 32%	14, 12%	<0.001
Type 2 diabetes mellitus, %	33, 25%	10, 9%	0.001
Dyslipidemia, %	35, 26%	22, 19%	0.205
Coronary heart disease, %	6, 5%	0, 0%	0.022
Cerebrovascular disease, %	1, 1%	1, 1%	0.908
Chronic kidney diseases, %	5, 4%	1, 1%	0.145
Waist circumference in cm	99.4±10.0	89.0±9.9	<0.001
Hip circumference in cm	104.9±9.3	98.3±11.2	<0.001
Platelets as 10^9/L	237±68	231±70	0.508
ALT in U/L	55 (31, 102)	61 (32, 109)	0.972
FPG, mmol/L	5.9±1.8	5.1±0.9	<0.001
HbA1c, %	6.37±1.69	5.64±0.70	<0.001
TG in mmol/L	1.90 (1.44, 2.62)	1.42 (1.06, 2.05)	<0.001
Total cholesterol in mmol/L	4.91±1.12	4.91±1.01	0.943
HDL-C in mmol/L	1.01±0.22	1.13±0.24	<0.001
Low-density lipoprotein-cholesterol in mmol/L	3.10±0.81	3.18±0.84	0.497
HOMA-IR	3.8 (2.6, 5.6)	2.4 (1.7, 3.5)	<0.001
eGFR in mL/min per 1.73m^2	107±17	108±17	0.702
CVD risk score	9 (5, 14)	4 (0, 10)	<0.001

ALT, alanine aminotransferase; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; HOMA-IR, homeostasis model assessment-insulin resistance; MetS, metabolic syndrome.

pared to the use of only one noninvasive measurement. Using these stepwise algorithms, the number of patients requiring liver biopsy was significantly reduced (Fig. 2), and liver biopsy could be reduced from 36% to 17% (p<0.001). Among these, FIB-4-MetDis had better diagnostic performance than FIB-4 alone (66%, p<0.001). It provided the highest accuracy (85%), highest positive likelihood ratio (5.92) and a high negative predictive value, which could avoid unnecessary liver biopsy (Table 4). Therefore, we recommend evaluating metabolic disorders after calculating the FIB-4 score for patients with hepatic steatosis found incidentally in the primary care unit (Fig. 3).

Discussion

In this study, we demonstrated the association between metabolic disorders and the severity of liver fibrosis in patients with NAFLD, and developed new algorithms combining metabolic disorders with noninvasive measurements to improve the diagnostic performance of advanced fibrosis. It is well known that liver biopsy is invasive, with an accompanying 1% risk of serious complications and an approximately 0.2% risk of mortality. FIB-4-MetDis could reduce the need for liver biopsy due to its high negative predictive value at the current study. Therefore, we recommend FIB-4-MetDis to screen advanced fibrosis in patients with NAFLD, which is available in most primary care units. The combination of metabolic disorders and noninvasive assessment is simple for clinicians to use to make a quick judgment.

The liver is the main organ that handles the excess burden of energy overload. NAFLD was even recognized as being among the spectrum of MetS. Recently, an international panel of experts suggested the nomenclature of metabolic dysfunction-associated fatty liver disease (i.e. MAFLD)[15] and issued guidelines[16] to characterize the disease and call attention to metabolic dysfunctions. In the current study, most of the patients (239/246, 97%) met the criteria of MAFLD. Compared to obesity or increased BMI, metabolic disorders may represent the most significant characteristic of NAFLD. We speculate that the severity of NAFLD, including clinical characteristics and pathological stages, reflects the severity of metabolic status in the liver. Previous studies revealed that as the number of metabolic abnormalities increased, the hepatic steatosis grades also increased in NAFLD,[3] which was similar to our findings. Thus, these metabolic disorders may act as predictors of the severity of NAFLD.

Raised FPG and insulin resistance are the most important features of metabolically unhealthy individuals and are

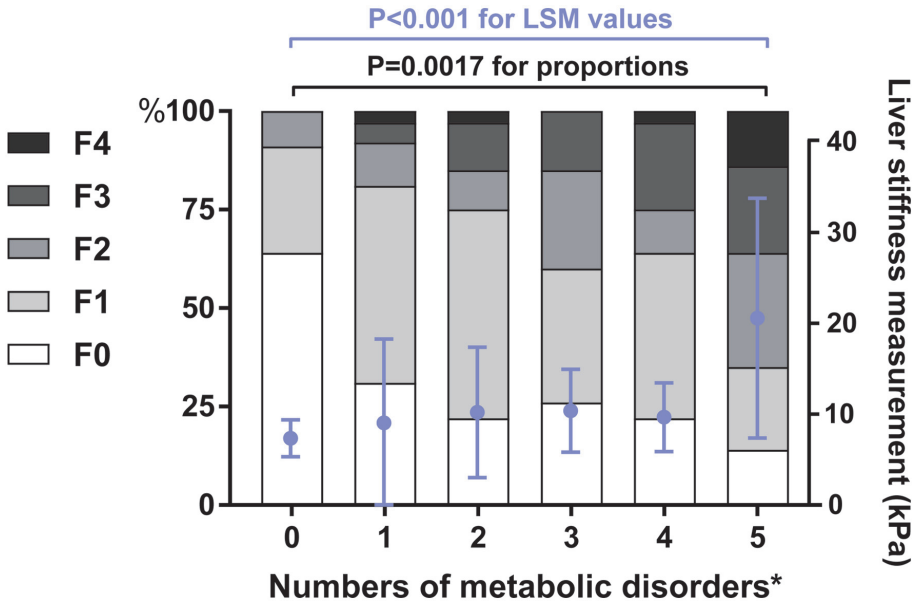

Fig. 1. Association between fibrosis stages and LSM with numbers of metabolic disorders. *Metabolic disorders referred to five components of metabolic disorders (IDF 2005): central obesity, raised blood pressure, reduced high-density lipoprotein cholesterol, raised triglyceride and raised fasting plasma glucose. LSM, liver stiffness measurement.

widespread in NAFLD patients.[17,18] NAFLD patients with diabetes and insulin resistance are at higher risk of developing advanced fibrosis and liver complications.[19,20] Insulin resistance also interacts with advanced fibrosis. Reduced glycogen synthesis and defects in glucose oxidation in cirrhotic patients further promote the development of impaired glucose tolerance and insulin resistance.[21] Among the current noninvasive models, other related indexes, such as blood glucose, hyperglycemia or diabetes, were also used to diagnose fibrosis according to the BARD score, NFS, FibroMeter and FIB-C3. Another risk factor for advanced fibrosis is reduced serum levels of HDL-C. Dyslipidemia is rather common in NAFLD patients.[22] The majority of hepatic fatty acids are from adipose tissue lipolysis, which is promoted by insulin resistance. HDL-C levels are also related to the severity of fibrosis.[23] Abnormal cholesterol metabolism could directly drive hepatic stellate cell activation, which promotes collagen secretion and fibrogenesis.[24] In addition, several lipid-regulating agents have been shown to improve liver fibrosis.[25] Although HDL-C is not often included in noninvasive tests, dyslipidemia also provides a clue for detecting fibrosis.

With the pandemic of metabolic-associated diseases, the prevalence of NAFLD is rapidly increasing in the past dec-

Table 2. Metabolic factors associated with advanced fibrosis

Variable	Univariate analysis		Multivariate analysis	
	OR, 95% CI	*p*	OR, 95% CI	*p*
Central obesity	0.705 [0.291, 1.706]	0.438		
Raised FPG	4.736 [2.221, 10.101]	<0.001	4.500 [2.083, 9.725]	<0.001
Raised BP	0.906 [0.452, 1.817]	0.781		
Raised TG	0.548 [0.271, 1.110]	0.095		
Reduced HDL-C	2.204 [0.954, 4.295]	0.066	2.241 [1.004, 5.002]	0.049

BP, blood pressure; CI, confidence interval; FPG, fasting plasma glucose; HDL-C, high density lipoprotein cholesterol-C; OR, odds ratio; TG, elevated triglyceride.

Table 3. Distribution of fibrosis stages in patients with different metabolic disorders

Metabolic disorders*	None	Either	Both	*p*
n	66	121	59	–
Significant fibrosis, %	11, 17%	34, 28%	31, 53%	<0.001
Advanced fibrosis, %	3, 5%	16, 13%	19, 32%	<0.001
Cirrhosis, %	1, 2%	2, 2%	3, 5%	0.319
LSM in kPa	7.3 (5.3, 9.5)	9.0 (6.6, 12.1)	10.0 (7.0, 14.2)	<0.001

*Reduced high-density lipoprotein cholesterol or raised fasting plasma glucose. LSM, liver stiffness measurement.

Table 4. Diagnostic performance of new algorithms combined with metabolic factors

Diagnostic algorithm	Accuracy, %	Sensitivity, %	Specificity, %	PPV, %	NPV, %	LR+	LR-	DOR	*p*
MetDis	82.52	92.11	80.77	46.67	98.25	4.79	0.10	49.0	<0.001[a]
MetDis-LSM	65.42	95.49	62.25	62.25	95.49	2.53	0.07	8.2	0.692[a]
LSM-MetDis	69.66	86.11	66.67	31.96	96.35	2.58	0.21	12.4	0.170[a]
MetDis-NFS	79.27	71.05	80.77	40.30	93.85	3.69	0.36	10.3	0.101
NFS-MetDis	84.02	48.78	91.13	52.63	89.81	5.5	0.56	9.8	0.003[b]
MetDis-FIB-4	80.08	81.58	79.81	42.47	95.95	4.04	0.23	17.5	<0.001[c]
FIB-4- MetDis	85.31	65.79	88.89	52.08	93.40	5.92	0.38	15.4	<0.001[c]

[a]Comparison of accuracy with LSM; [b]Comparison with NFS; [c]Comparison with FIB-4. MetDis-LSM, evaluating metabolic disorders (reduced HDL-C or raised FPG) as a first step, and use LSM as second step; LSM-MetDis is opposite. MetDis-NFS, evaluating metabolic disorders as a first step, and use NFS as second step; NFS-MetDis is opposite. MetDis-FIB-4, evaluating metabolic disorders as a first step, and use FIB-4 as second step; FIB-4- MetDis is opposite. DOR, diagnostic odds ratio; LR+, positive likelihood ratio; LR-, negative likelihood ratio; NPV, negative predictive value; PPV, positive predictive value.

ades. An important issue concerning clinical practice is the diagnostic performance of noninvasive measurements for advanced fibrosis. Serum-based models are more available in primary medical centers. The aspartate aminotransferase to platelet ratio index (commonly known as APRI), FIB-4 and BARD score indexes could be collected from routine tests and are easily calculated. However, these models lack sufficient sensitivity to rule in advanced fibrosis.[26] Other serum-based models consisting of special indicators (i.e. Pro-C3, PIIINP, or TIMP-1) and patents (e.g., FibroMeter) have limitations in their application due to accessibility. Image-based noninvasive measurements are more sensitive in detecting advanced fibrosis. These tests have a rather high negative predictive value for ruling out advanced fibrosis.[27] In this situation, sequential combinations of noninvasive measurements could provide a solution.

Our study has the strengths of a well-established design with all the data monitored by experienced groups. The tissue biopsy slices from each patient were reread by pathologists, which guaranteed an accurate evaluation of the characteristics of NAFLD and exclusion of other liver diseases. However, there were still several limitations. First, the sample size was relatively small. Patients in this study were all collected from tertiary medical centers. The information of the patients was collected mainly from in-patient medical reports within 6 months to reduce recall bias. Complete data ensured the quality of the research. Given an expanded sample size, the power of the conclusion could be enhanced. Second, we did not follow-up with the patients to observe long-term prognosis and liver-related events. It

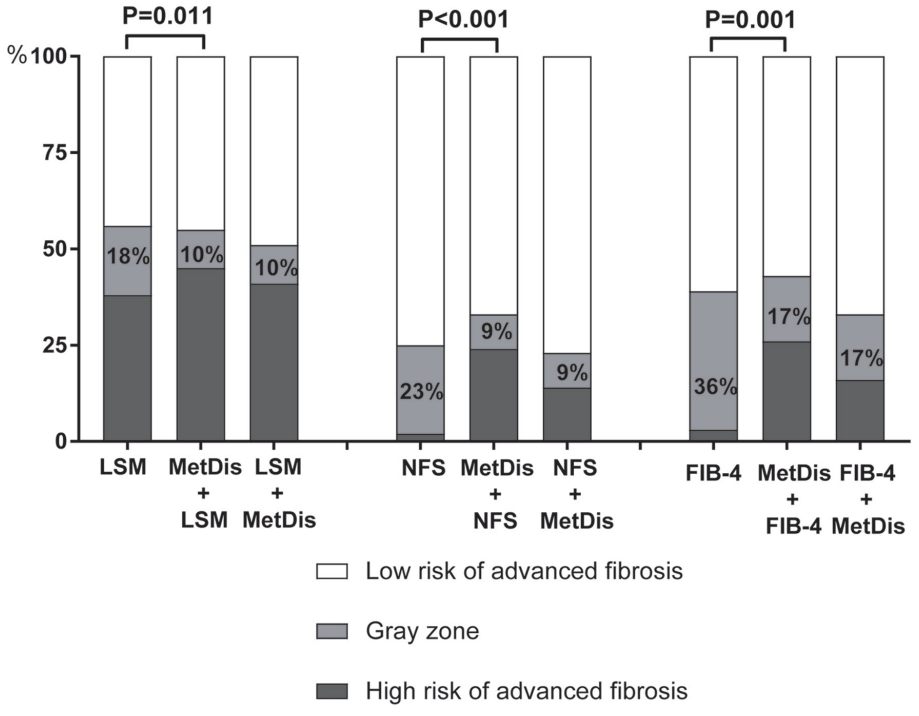

Fig. 2. Distribution of patients when using different diagnostic algorithms. Rule-in, patients met the criteria to diagnose advanced fibrosis according to published cut-off; Rule-out, patients met the criteria to exclude advanced fibrosis according to published cut-off; Gray zone, undiagnosed patients in the middle of the criteria, needed to be further examined, for instance, liver fibrosis. FIB-4, fibrosis-4 score; LSM, liver stiffness measurement; MetDis, metabolic disorders; NFS, NAFLD fibrosis socre.

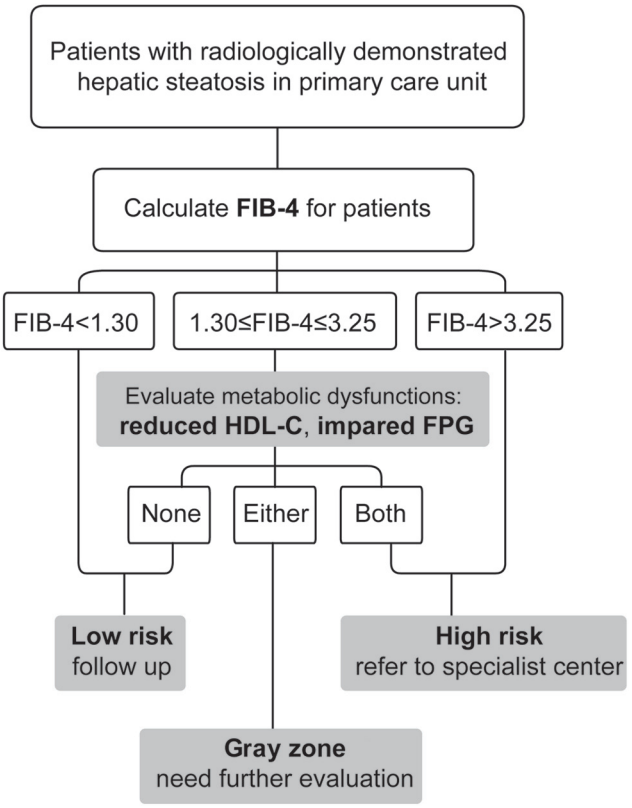

Fig. 3. Diagnostic flow-chart to monitor risk of advanced fibrosis. Combining FIB-4 test and metabolic disorders in monitoring advanced fibrosis. Low risk: follow-up every 2 years, according to EASL guideline; Gray zone: further evaluation included other non-invasive tests, liver stiffness measurement, magnetic resonance elastography, even liver biopsy, or specialist referral; High risk: refer to specialist to evaluate disease severity and identify other potential liver diseases. FIB-4, fibrosis-4 score; FPG, fasting plasma glucose; HDL-C, high-density lipoprotein cholesterol.

has been reported that overweight status and T2DM are key determinants of fibrosis progression.[28] Thus, in further studies, we could focus on the role of raised FPG and reduced HDL-C in the development of liver fibrosis and cirrhosis.[29]

In conclusion, metabolic disorders contributed to the severity of fibrosis in NAFLD patients, which should be taken into consideration during diagnosis and management. New combinations of metabolic disorders with noninvasive measurements provided a more accurate diagnosis for advanced fibrosis. With further validation in external cohorts, this algorithm could be recommended as a first-line screening of advanced fibrosis in primary care units.

Acknowledgments

We would like to thank Xiao-Jin Wang and Cui-Hua Huang for their great help in the study. The language editing service was provided by MIMS Shanghai, Ltd, funded by Sanofi.

Funding

This study was funded by Sanofi (China) Investment Co., Ltd and the National Key R&D Program of China (No. 2017YFC090890).

Conflict of interest

BP is an employee of Sanofi. None of the other authors have any potential or real conflicts of interest to declare. The authors did not receive any payment for authoring this publication.

Author contributions

Study concept and design (BP, JGF), acquisition of data (FPH, JJC, HD, JPS, CYZ, YQM, ZSZ, YJZ, FSD, RDZ, QD, JS, RXY, BHZ, JGF), analysis and interpretation of data (YWS, JGF), drafting of the manuscript (YWS, JGF), critical revision of the manuscript for important intellectual content (JGF). All authors confirmed critical revision of the manuscript for important intellectual content.

Data sharing statement

No additional data are available.

References

[1] 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(1):73–84. doi:10.1002/hep.28431.
[2] Lin S, Huang J, Wang M, Kumar R, Liu Y, Liu S, *et al*. Comparison of MAFLD and NAFLD diagnostic criteria in real world. Liver Int 2020;40:2082–2089. doi:10.1111/liv.14548.
[3] Jinjuvadia R, Antaki F, Lohia P, Liangpunsakul S. The Association between nonalcoholic fatty liver disease and metabolic abnormalities in the United States population. J Clin Gastroenterol 2017;51:160–166. doi:10.1097/MCG.0000000000000666.
[4] Sookoian S, Pirola CJ. Systematic review with meta-analysis: the significance of histological disease severity in lean patients with nonalcoholic fatty liver disease. Aliment PharmacolTher 2018;47:16–25. doi:10.1111/apt.14401.
[5] Gutierrez-Grobe Y, Juarez-Hernandez E, Sanchez-Jimenez BA, Uribe-Ramos MH, Ramos-Ostos MH, Uribe M, *et al*. Less liver fibrosis in metabolically healthy compared with metabolically unhealthy obese patients with non-alcoholic fatty liver disease. Diabetes Metab 2017;43:332–337. doi:10.1016/j.diabet.2017.02.007.
[6] Feldman A, Eder SK, Felder TK, Kedenko L, Paulweber B, Stadlmayr A, *et al*. Clinical and metabolic characterization of lean caucasian subjects with non-alcoholic fatty liver. Am J Gastroenterol 2017;112:102–110. doi:10.1038/ajg.2016.318.
[7] Dulai PS, Singh S, Patel J, Soni M, Prokop LJ, Younossi Z, *et al*. Increased risk of mortality by fibrosis stage in nonalcoholic fatty liver disease: Systematic review and meta-analysis. Hepatology 2017;65:1557–1565. doi:10.1002/hep.29085.
[8] Bedossa P. Utility and appropriateness of the fatty liver inhibition of progression (FLIP) algorithm and steatosis, activity, and fibrosis (SAF) score in the evaluation of biopsies of nonalcoholic fatty liver disease. Hepatology 2014;60:565–575. doi:10.1002/hep.27173.
[9] European Association for the Study of the Liver (EASL); European Association for the Study of Diabetes (EASD); European Association for the Study of Obesity (EASO). EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. J Hepatol 2016;64(6):1388–1402. doi:10.1016/j.jhep.2015.11.004.
[10] Xu ZJ, Shi JP, Yu DR, Zhu LJ, Jia JD, Fan JG. Evaluating the relationship between metabolic syndrome and liver biopsy-proven non-alcoholic steatohepatitis in China: a multicenter cross-sectional study design. Adv Ther 2016;33:2069–2081. doi:10.1007/s12325-016-0416-4.
[11] Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, *et al*. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Circulation 2005;112(17):2735–2752. doi:10.1161/CIRCULATIONAHA.105.169404.
[12] Angulo P, Hui JM, Marchesini G, Bugianesi E, George J, Farrell GC, *et al*. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. Hepatology 2007;45:846–854. doi:10.1002/hep.21496.
[13] Sterling RK, Lissen E, Clumeck N, Sola R, Correa MC, Montaner J, *et al*. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. Hepatology 2006;43:1317–1325. doi:10.1002/hep.21178.
[14] Wong VW, Vergniol J, Wong GL, Foucher J, Chan HL, Le Bail B, *et al*. Di-

agnosis of fibrosis and cirrhosis using liver stiffness measurement in non-alcoholic fatty liver disease. Hepatology 2010;51:454–462. doi:10.1002/hep.21178.
[15] Eslam M, Newsome PN, Sarin SK, Anstee QM, Targher G, Romero-Gomez M, *et al*. A new definition for metabolic dysfunction-associated fatty liver disease: an international expert consensus statement. J Hepatol 2020;73(1):202–209. doi:10.1016/j.jhep.2020.03.039.
[16] Eslam M, Sanyal AJ, George J. Toward more accurate nomenclature for fatty liver diseases. Gastroenterology 2019;157:590–593. doi:10.1053/j.gastro.2019.05.064.
[17] Mantovani A, Petracca G, Beatrice G, Tilg H, Byrne CD, Targher G. Non-alcoholic fatty liver disease and risk of incident diabetes mellitus: an updated meta-analysis of 501 022 adult individuals. Gut 2021;70(5):962–969. doi:10.1136/gutjnl-2020-322572.
[18] Chitturi S, Abeygunasekera S, Farrell GC, Holmes-Walker J, Hui JM, Fung C, *et al*. NASH and insulin resistance: insulin hypersecretion and specific association with the insulin resistance syndrome. Hepatology 2002;35:373–379. doi:10.1053/jhep.2002.30692.
[19] Bertot LC, Jeffrey GP, de Boer B, MacQuillan G, Garas G, Chin J, *et al*. Diabetes impacts prediction of cirrhosis and prognosis by non-invasive fibrosis models in non-alcoholic fatty liver disease. Liver Int 2018;38:1793–1802. doi:10.1111/liv.13739.
[20] Aller R, Sigüenza R, Pina M, Laserna C, Antolín B, Burgueño B, *et al*. Insulin resistance is related with liver fibrosis in type 2 diabetic patients with non-alcoholic fatty liver disease proven biopsy and mediterranean diet pattern as a protective factor. Endocrine 2020;68:557–563. doi:10.1007/s12020-020-02268-7.
[21] Petrides AS, Vogt C, Schulze-Berge D, Matthews D, Strohmeyer G. Pathogenesis of glucose intolerance and diabetes mellitus in cirrhosis. Hepatology 1994;19:616–627. doi:10.1002/hep.1840190312.
[22] Chatrath H, Vuppalanchi R, Chalasani N. Dyslipidemia in patients with non-alcoholic fatty liver disease. Semin Liver Dis 2012;32:22–29. doi:10.1055/s-0032-1306423.
[23] Klisic A, Abenavoli L, Fagoonee S, Kavaric N, Kocic G, Ninić A. Older age and HDL-cholesterol as independent predictors of liver fibrosis assessed by BARD score. Minerva Med 2019;110:191–198. doi:10.23736/S0026-4806.19.05978-0.
[24] Wang X, Zheng Z, Caviglia JM, Corey KE, Herfel TM, Cai B, *et al*. Hepatocyte TAZ/WWTR1 promotes inflammation and fibrosis in nonalcoholic steatohepatitis. Cell Metab 2016;24:848–862. doi:10.1016/j.cmet.2016.09.016.
[25] Takeshita Y, Takamura T, Honda M, Kita Y, Zen Y, Kato K, *et al*. The effects of ezetimibe on non-alcoholic fatty liver disease and glucose metabolism: a randomised controlled trial. Diabetologia 2014;57:878–890. doi:10.1007/s00125-013-3149-9.
[26] Petta S, Wong VW, Cammà C, Hiriart JB, Wong GL, Vergniol J, *et al*. Serial combination of non-invasive tools improves the diagnostic accuracy of severe liver fibrosis in patients with NAFLD. Aliment PharmacolTher 2017;46:617–627. doi:10.1111/apt.14219.
[27] Loomba R. Role of imaging-based biomarkers in NAFLD: recent advances in clinical application and future research directions. J Hepatol 2018;68:296–304. doi:10.1016/j.jhep.2017.11.028.
[28] Schuppan D, Surabattula R, Wang XY. Determinants of fibrosis progression and regression in NASH. J Hepatol 2018;68:238–250. doi:10.1016/j.jhep.2017.11.012.
[29] Ren TY, Fan JG. What are the clinical settings and outcomes of lean NAFLD? Nat Rev Gastroenterol Hepatol 2021. doi:10.1038/s41575-021-00433-5.