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Advanced fibrosis associates with atherosclerosis in subjects with nonalcoholic fatty liver disease

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ABSTRACT

Objective: Nonalcoholic fatty liver (NAFLD) with advanced fibrosis usually has a deteriorated prognosis, which was mainly attributed to cardiovascular cause. We investigated whether advanced fibrosis assessed by noninvasive fibrosis markers was associated with subclinical atherosclerosis in NAFLD patients.

Methods: A total of 2550 participants with ultrasound confirmed NAFLD from a community based population study were included in the present analysis. NAFLD fibrosis score (NFS) derived from available parameters was calculated to assess severity of fibrosis of the NAFLD patients. The NAFLD patients with a NFS > 0.676 indicated of presence of advanced fibrosis. The carotid intima-media thickness (CIMT), carotid plaques and brachial-ankle pulse wave velocity (ba-PWV) were used as the indicators of early atherosclerosis.

Results: NAFLD patients with advanced fibrosis had higher CIMT and ba-PWV, compared with those without fibrosis (CIMT: 0.65 versus 0.57 mm; ba-PWV: 1884 versus 1535 cm/s, both $p < 0.0001$). Participants with advanced fibrosis were more likely to have higher homeostasis model assessment of insulin resistance index (HOMA_IR, 3.28 versus 2.45, $p < 0.0001$). After adjusting the confounders, participants with advanced fibrosis associated with 1.98-folds increased risk for elevated CIMT, 2.28-folds increased risk for present carotid plaque and 2.68-folds increased risk for arterial stiffness, respectively, as compared to participants without fibrosis. After further adjustment for HOMA_IR, the positive associations did not appreciably change.

Conclusion: Advanced fibrosis indicated by NFS was positively associated with CIMT, presence of carotid plaque and arterial stiffness in the NAFLD patients, independent of conventional cardiometabolic risk factors and insulin resistance.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) indicates a spectrum of liver diseases that encompass simple steatosis, fatty infiltration plus inflammation (NASH), fibrosis and ultimately cirrhosis. With an increase presence of NAFLD global widely, it has posed great burden on public health. Fatty liver has been considered as a risk factor of cardiovascular events, and currently the grade of NAFLD determines the progressive cardiovascular risk [1,2]. Simple steatosis is fairly benign and reversed [3,4], however, NAFLD progressing to NASH or advance fibrosis has a deteriorated prognosis [5]. The gold standard to confirm presence and severity of NAFLD fibrosis depends on the utilization of liver biopsy, which was not

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universally accepted by patients in clinic practice.

Recently, a growing number of studies were performed trying to explore the clinic value of the non-invasive scores for NAFLD fibrosis [6]. NAFLD fibrosis score (NFS) has been validated in 13 studies with more than 3000 patients [7], and it incorporates age, body mass index (BMI), hyperglycemia, blood platelet count, serum albumin and aspartate aminotransferase/alanine aminotransferase ratio (AST/ALT), which presents great accuracy for diagnosis of advanced fibrosis [8]. This score has been recommended to be applied in clinical practice with 97% specificity for confirming advanced fibrosis at the cut-off point of 0.676 [9]. Results derived from the National Health and Nutrition Examination Survey (NHANES) have shown that advanced fibrosis determined by non-invasive fibrosis panels is a significant predictor of mortality caused by cardiovascular disease (CVD) in patients with NAFLD, independent of other known risk factors [10]. To our best knowledge, studies exploring the association between NAFLD fibrosis and subclinical arterial vascular disease in community-based ultrasonography-confirmed NAFLD patients are limited. This study aims to evaluate the association of advanced fibrosis assessed by NFS with markers of subclinical arterial vascular disease such as carotid intima-media thickness (CIMT), presence of carotid plaques and arterial stiffness measured by brachial-ankle pulse wave velocity (ba-PWV).

2. Patients and methods

2.1. Subjects and study design

The participants were from a community-based cross-sectional survey, which was conducted in Jiading district, Shanghai, China, from March to August, 2010. The details of the study, including design, sampling extracting, eligibility criteria, items detected, information collected, have been described elsewhere [11]. A total of 10,375 inhabitants aged 40 years or older were recruited to take part in this survey. All participants were undergone abdominal ultrasonic examination. Of those, participants with a history of known liver disease such as viral or autoimmune hepatitis, liver cancer, or cirrhosis (n = 975), or participants abusing alcohol (alcohol consumption ≥140 g/week in men or ≥70 g/week in women, n = 863) were excluded. Further excluding those participants with missing information of atherosclerosis parameters such as CIMT or ba-PWV (n = 101), or NFS parameters including BMI, ALT, AST, blood platelet count, serum albumin and glucose levels (n = 155), a total of 8281 participants remained. Among them, 2550 participants suffered from NAFLD determined by hepatic ultrasonic examination and were ultimately included in the present analysis.

The study protocol was approved by the Institutional Review Board of the Ruijin Hospital, the Shanghai Jiao Tong University School of Medicine. All participants gave their written consents.

2.2. Clinical and laboratory evaluation

A standard questionnaire was performed to obtain the information on demographic characteristics, lifestyles, history of diseases and medication usage with face-to-face interviews by trained investigators. Participants' body weight and height were obtained in light clothes and bare feet to the nearest 0.1 kg and 0.1 cm, respectively. BMI was calculated according to weight in kilograms divided by square of height in meters. Waist circumference (WC) was measured at the level of the umbilicus with the patient in a standing position. Blood pressure was obtained on the non-dominant arm at a seated position with an automated electronic sphygmomanometer (OMRON Model HEM-752 FUZZY' Omron Co., Dalian, China) three times consecutively with 1-min interval, after at least 10-min rest. The average values of the three readings were used. Regular consume of cigarettes in the past 6 months was defined as current smoker. In line with it, regular consume of alcohol in the past 6 months was defined as current drinker. Fasting venous blood samples were collected after at least 10-h fast. Serum fasting triglycerides (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), ALT, AST, gamma-glutamyl transpeptidase (GGT) and serum albumin were measured on the autoanalyser (Modular E170, Roche). White blood cell (WBC), platelet count (PLT), and hemoglobin were measured on an automated cell counter (Hematology analyzer 120, ABX, France). A 75 g oral glucose tolerance test (OGTT) was conducted to collect two points (0 and 2 h) blood samples. Fasting blood glucose (FBG) and 2-h post-load glucose levels were measured by glucose oxidase method on an autoanalyser (Modular P800, Roche). Serum insulin was measured by using an electrochemiluminescence assay (Modular E170, Roche). The homeostasis model assessment of insulin resistance index (HOMA_IR) was calculated as fasting insulin (μIU/ml) × fasting glucose (mmol/L)/22.5.

According to 1999 World Health Organization (WHO) criteria, diabetes mellitus was defined as FBG levels of 7.0 mmol/L or higher, or a 2-h post-load glucose levels of 11.1 mmol/L or higher, or taking antidiabetic medications or insulin injection. Impaired fasting glycemia was defined as FBG between 6.1 mmol/L and 7.0 mmol/L, and 2-h post-load glucose levels less than 7.8 mmol/L. Hypertension was defined as systolic blood pressure of 140 mmHg or higher, or diastolic blood pressure of 90 mmHg or higher, or using antihypertensive drugs. Prior CVD referred to a self-reported history of myocardial infarction, coronary heart disease and stroke.

2.3. NAFLD and advanced fibrosis

Hepatic ultrasonic examination was performed by two specialists on ultrasonography who were blind to other data with a high-resolution B-mode tomographic ultrasound system (Esaote Biomedica SpA, Italy) equipped with a 3.5-MHz probe. Definition of NAFLD was based on the presence of at least two of the following three abnormal findings according to 2010 Chinese Guidelines on Diagnosis and Treatment of NAFLD [12]: diffusely increased echogenicity of the liver relative to the kidney, ultrasound beam attenuation, and poor visualization of intrahepatic structures, and the above caused by other than alcohol abuse and known liver disease.

In patients with NAFLD, NFS was used to assess severity of fibrosis. NFS was calculated according to the published formula: NFS = −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 × AST/ALT − 0.013 × platelet count (×10^9/L) − 0.66 × serum albumin (g/dL) [8]. Two cut-off points were select to categorize participants with NAFLD into three groups: those with high NFS (NFS > 0.676), intermediate NFS (NFS: −1.455 ~ 0.676), and low NFS (NFS < −1.455). Patients with high NFS have 97% specificity to identify the presence of advanced fibrosis, whereas patients with low NFS have 90% sensitivity to exclude advanced fibrosis [8].

2.4. Subclinical atherosclerosis panels

CIMT and carotid plaque were measured using a high-resolution B-mode tomographic ultrasound system (Esaote Biomedica SpA, Italy), with a linear 7.5-MHz transducer by one experienced sonographer. The operator measured CIMT on the far wall of the common carotid arteries, 1.5 cm proximal to the bifurcation. The distance from the leading edge of the first echogenic line to that of the second echogenic line at the end of diastole was taken for CIMT.

The average of the right and left common CIMT was used for analysis. Participants in the upper quartile of CIMT (≥0.7 mm [13]) were referred as having elevated CIMT. In addition, the extracranial segments of the left and right carotid arteries (common carotid, bulb, internal carotid, external carotid) were extensively scanned for the presence of carotid plaque, which was defined as focal protrusion (IMT with a thickness exceeding that of the surrounding wall by 50%).

The participants were measured the ba-PWV on Colin VP-1000 (Model BP203RPE II, form PWV/ABI) after 10–15 min' rest. Pulse waves were obtained simultaneously with cuffs placed on the right/left upper arm and the right/left ankle. The distance from right/left upper arm to right/left ankle was corrected for its time delay difference to obtain the ba-PWV. The average value of the right and left ba-PWV was calculated for analysis. Upper quartile of average ba-PWV (≥1799 cm/s) was regarded as arterial stiffness [11].

2.5. Statistical analysis

Statistical analysis was performed on SAS 9.3 (SAS Institute, Cary, NC). Normally distributed variables were presented as means with standard deviations (SDs), whereas skewed distributed variables were presented as geometrical means and 95% confidence intervals (CIs) and analyzed after logarithmic transformation. The differences of continuous variables across groups of different severity of fibrosis were tested by one-way analysis of variance (ANOVA), and the dichotomous variables with chi-square tests.

Taken elevated CIMT, presence of carotid plaque and arterial stiffness as dichotomous dependent variables, we conducted logistic regression analysis to explore the associations between severity of fibrosis and atherosclerosis in unadjusted and multivariate adjusted models. In multivariate model, covariates included age, sex, smoking and drinking status, obesity, diabetes mellitus and hypertension, prior CVD history, serum lipids and HOMA_IR. A two-sided p value less than 0.05 was considered as statistical significance.

3. Results

3.1. Characteristics of NAFLD participants

A total of 2550 participants were confirmed as NAFLD patients by ultrasonography, accounting for 30.8% of all participants. Demographic and clinical characteristics of participants with NAFLD were summarized in Table 1. According to the cut-off points of NFS at −1.455, 0.676 separately, 103 participants had advanced fibrosis, and 1226 participants were excluded to have advanced fibrosis. As expected from the component variables of the score, advanced fibrosis was associated with older age. Participants with advanced fibrosis were more likely to have higher BMI, WC, systolic blood pressure (SBP), glucose levels and HOMA_IR. Moreover, NAFLD participants with advanced fibrosis had higher prevalence of diabetes, hypertension and prior CVD history. Advanced fibrosis was negatively associated with serum fasting concentrations of TC and

Table 1
Characteristics of participants with NAFLD according to NFS levels.

	Low NFS (<−1.455)	Intermediate NFS (−1.455–0.676)	High NFS (>0.676)	p for trend
n	1226	1221	103	
Age, y	54.5 ± 7.3	61.6 ± 8.2	71.1 ± 8.2	<0.0001
Male, n (%)	401 (32.7)	376 (30.8)	30 (29.1)	0.25
BMI, kg/m^2	26.9 ± 2.7	27.9 ± 3.0	30.1 ± 4.1	<0.0001
WC, cm	87.3 ± 7.2	89.9 ± 7.9	94.9 ± 9.2	<0.0001
SBP, mm/Hg	143 ± 19	149 ± 20	158 ± 23	<0.0001
DBP, mm/Hg	86 ± 10	85 ± 10	82 ± 11	<0.0001
Current smoker, n (%)	217 (17.7)	164 (13.4)	6 (5.8)	<0.0001
Current drinker, n (%)	32 (2.6)	33 (2.7)	4 (3.9)	0.61
Physical activity, MET-h/week	17 (0–90)	21 (0–84)	21 (0–63)	0.95
TG[a], mmol/L	1.87 (0.86–4.90)	1.89 (0.89–5.01)	1.70 (0.92–4.51)	0.35
TC, mmol/L	5.58 ± 1.11	5.45 ± 1.04	5.28 ± 0.92	0.002
LDL-C, mmol/L	3.37 ± 0.91	3.27 ± 0.91	3.08 ± 0.77	0.006
HDL-C, mmol/L	1.19 ± 0.27	1.20 ± 0.27	1.26 ± 0.27	0.40
FBG, mmol/L	5.8 ± 1.7	6.3 ± 2.1	6.5 ± 2.1	<0.0001
2h-BG, mmol/L	8.8 ± 4.4	11.4 ± 5.2	12.7 ± 5.6	<0.0001
HOMA-IR[a]	2.45 (1.00–6.06)	2.82 (1.17–7.63)	3.28 (1.33–10.50)	<0.0001
WBC, × 10^9/L	6.4 ± 1.5	6.0 ± 1.4	5.6 ± 1.4	<0.0001
Hemoglobin, g/L	143 ± 13	141 ± 13	138 ± 13	0.001
PLT, × 10^9/L	260 ± 55	196 ± 44	143 ± 41	<0.0001
Serum albumin, g/L	49.3 ± 2.3	48.8 ± 2.3	47.3 ± 2.6	<0.0001
Serum ALT, IU/L	24 (12–60)	23 (12–65)	21 (10–66)	0.06
Serum AST, IU/L	22 (15–41)	23 (15–49)	24 (16–66)	<0.0001
Serum GGT, IU/L	29 (13–91)	28 (13–87)	26 (13–132)	0.57
CIMT, mm	0.57 ± 0.09	0.60 ± 0.11	0.65 ± 0.09	<0.0001
ba-PWV, cm/s	1535 ± 363	1693 ± 410	1884 ± 462	<0.0001
Diabetes mellitus, n (%)	234 (19.1)	542 (44.4)	57 (55.3)	<0.0001
Hypertension, n (%)	829 (67.6)	935 (76.6)	92 (89.3)	<0.0001
Self-reported CVD history, n (%)	77 (6.3)	147 (12.0)	22 (21.4)	<0.0001

Data are presented as mean ± standard deviation (SD) or median (95% confidence interval) or number (proportion). p for trend across NFS levels was analyzed by one-way analysis of variance (ANOVA) for continuous variables and chi-square test for categorical variables.
Abbreviations: NAFLD, nonalcoholic fatty liver disease; NFS, NAFLD fibrosis score; BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; MET, metabolic equivalent; TG, triglycerides; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; FBG, fasting blood glucose; 2h-BG, 2 h postprandial glucose; HOMA-IR, homeostasis model assessment of insulin resistance; WBC, white blood cell; PLT, platelet count; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transpeptidase; CIMT, carotid intima-media thickness; ba-PWV, brachial ankle pulse wave velocity; CVD, cardiovascular disease.
[a] Skewed variables were log-transformed before analysis.

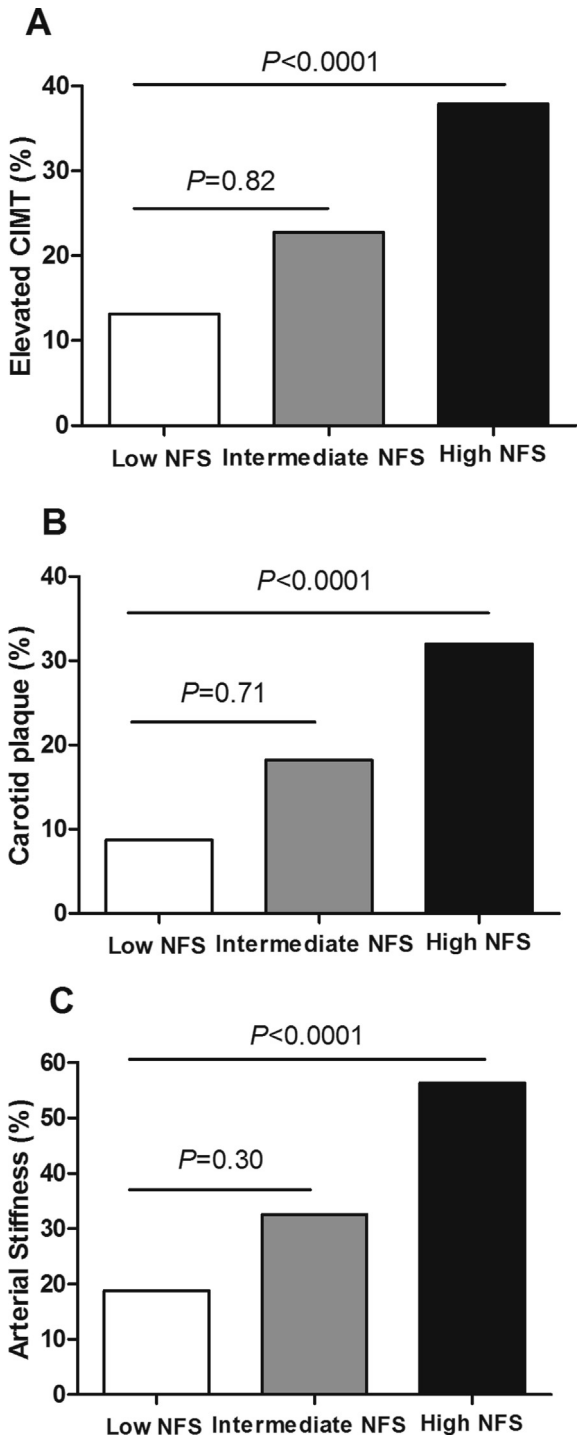

Fig. 1. Prevalence of elevated CIMT, carotid plaque, and arterial stiffness according to NFS levels.

LDL-C, and was positively associated with HDL-C. Furthermore, the CIMT and ba-PWV increased with NFS levels. For liver enzymes, advanced fibrosis was positively associated with AST, however, no statistically significant relationship with ALT and GGT.

3.2. Advanced NAFLD fibrosis was associated with prevalence of atherosclerosis

As shown in Fig. 1, among participants with NAFLD, prevalence of elevated CIMT was 13.1%, 22.8%, and 37.9% across the three NFS levels. Patients with advanced fibrosis had a higher risk of having elevated CIMT, compared to patients without advanced fibrosis ($p < 0.0001$). In parallel, the prevalence of carotid plaque was 8.7%, 18.2%, and 32.0%, and the arterial stiffness was 18.8%, 32.5%, and 56.3% across NFS levels, respectively. Patients with advanced fibrosis had a greater risk of prevalence of carotid plaque and arterial stiffness ($p < 0.0001$). Further partial correlation and multiple linear regression analyses were performed to explore the relationship between NFS and continuous subclinical atherosclerosis variables (CIMT and ba-PWV). Pearson's correlation analyses revealed that NFS was significantly correlated with CIMT ($r = 0.24$, p value < 0.0001) and ba-PWV ($r = 0.24$, p value < 0.0001). After performing multiple linear regression analyses, we found that NFS was positively correlated with CIMT and ba-PWV controlling for age, sex, smoking and drinking statuses, physical activity. The standardized regression coefficient of NFS was 0.07 ($p = 0.0018$) with CIMT and was 0.08 ($p = 0.0005$) with ba-PWV. However, NFS only moderately increased the risk for elevated CIMT and carotid plaque as compared with the classical risk factors such as age, sex, LDL-c, presence of diabetes and hypertension by multivariate analysis (Supplemental Tables 1 and 2). NFS also increased the risk for arterial stiffness, which was comparable with age, serum triglyceride, and presence of diabetes.

3.3. The odds ratios (ORs) of atherosclerosis of NAFLD participants with advanced fibrosis

Taken elevated CIMT, presence of carotid plaque and arterial stiffness as dependent variables respectively, unadjusted and further multivariable adjusted logistic regression models were performed to evaluate impact of advanced fibrosis on atherosclerosis. In unadjusted model, compared to group without advanced fibrosis, presence of advanced fibrosis associated with a 303% increased risk for elevated CIMT (OR, 4.03; 95%CI: 2.62–6.20; p for trend <0.0001), a 398% increased risk of prevalence of carotid plaque (OR, 4.98; 95%CI: 3.15–7.89; p for trend <0.0001), and a 456% increased risk for prevalence of arterial stiffness (OR, 5.56; 95%CI: 3.67–8.41; p for trend <0.0001). After further adjusted for age, sex, smoking and drinking status, disease of obesity, diabetes, hypertension, CVD history, lipid profile and WBC, the presence of advanced fibrosis was still associated with elevated CIMT, presence of carotid plaque, and arterial stiffness. Advanced fibrosis associated with 2.02-fold increased risk for elevated CIMT, 2.21-fold for carotid plaque and 2.37-fold for arterial stiffness, as compared to NAFLD participants without advanced fibrosis (Table 2). Further adjustment for HOMA_IR, the results did not change appreciably.

4. Discussion

In the present study, we found that NAFLD patients with advanced fibrosis related to a high risk of having elevated CIMT, presence of carotid plaque and arterial stiffness, independent of conventional metabolic factors and prior CVD history and insulin resistance.

NAFLD is well regarded as the manifestation of metabolic syndrome in liver. Due to a fast conversation of lifestyle occurred in the past decades, the prevalence of NAFLD is increasing sharply [14]. Abdominal ultrasonography is recommended as the first-line imaging modality [15] in demonstrating fatty liver, with a reported sensitivity greater than 80% and specificity greater than 90% [16,17]. The prevalence of NAFLD confirmed by abdominal ultrasonography in our population achieved a proportion of 30.8%, approaching to the proportion reported in developed countries [18]. NAFLD was progressively across simple steatosis to NASH, fibrosis, and finally hepatic carcinoma. Liver biopsy has been well recognized as the most

Table 2
Associations of NFS with elevated CIMT, presence of carotid plaque and arterial stiffness among participants with NAFLD.

	Unadjusted			Multivariate adjusted		
	OR	95% CI	*p* for trend	OR	95% CI	*p* for trend
Elevated CIMT						
Low NFS	1.00			1.00		
Intermediate NFS	1.95	1.58–2.41	<0.0001	1.25	0.91–1.72	
High NFS	4.03	2.62–6.20		2.02	1.11–3.69	0.04
Presence of carotid plaque						
Low NFS	1.00			1.00		
Intermediate NFS	2.35	1.84–3.01		1.42	0.99–2.03	
High NFS	4.98	3.15–7.89	<0.0001	2.21	1.16–4.21	0.01
Arterial stiffness						
Low NFS	1.00			1.00		
Intermediate NFS	2.08	1.72–2.50		1.23	0.92–1.64	
High NFS	5.56	3.67–8.41	<0.0001	2.37	1.33–4.21	0.01

Data are odds ratio (OR), 95% confidence interval (CI). Multivariate adjustment included age, sex, smoking and drinking status, physical activity, obesity, diabetes, hypertension, history of cardiovascular disease, low density lipoprotein cholesterol, triglycerides, total cholesterol, high density lipoprotein cholesterol, platelet count, white blood cell count.

reliable approach for identifying the presence of fibrosis in patients with NAFLD, but its deficiency of high cost, sampling error, and procedure-related morbidity and mortality limits its application in clinic. Therefore, there has been intense interest in developing non-invasive biomarkers for identifying advanced fibrosis in patients with NAFLD. The NFS is derived from six common available variables including age, BMI, hyperglycemia, platelet count, albumin and AST/ALT ratio. In a meta-analysis of 13 studies consisting of more than 3000 NAFLD patients, NFS has a good predictive value for advanced fibrosis and a score of < -1.455 has 90% sensitivity and 60% specificity to exclude advanced fibrosis; whereas a score of >0.676 has 67% sensitivity and 97% specificity to identify the presence of advanced fibrosis [7]. According to the American Association for the Study of Liver Diseases (AASLD), American College of Gastroenterology (ACG) and American Gastroenterological Association (AGA) 2012 guideline, NFS was recommended to identify patients who are at risk of advanced fibrosis [9]. Adopting 0.676 as the cut-off point, the high probability of advanced fibrosis accounted for 4% of the participants in the present study, which was comparable with the proportion of 4.2% in NHANES' population [10].

The increased risk of cardiovascular disease in patients with NAFLD drew particular attentions in the past decade [19]. Considering its great association with traditional cardiovascular risks, inconsistence existed on whether NAFLD itself or the concurrent risk factors attributed to the progression of atherosclerosis, even cardiovascular diseases. The underlying chronic process of cardiovascular disease is atherosclerosis which is present in early and late stage vascular disease. CIMT, carotid plaque, ba-PWV and coronary artery calcification are well regarded noninvasive parameters to detect atherosclerosis. It has been showed that NAFLD defined by ultrasonography was positively associated with coronary artery calcification independently of demographics, health behaviors and visceral adipose tissue [20]. However, across the spectrum of NAFLD, simple fatty liver is generally thought to be “non progressive”, differently from progressive NASH and fibrosis. It was reported that simple hepatic steatosis played a scarce role in atherosclerosis [21]. On the other side, some prospective studies in NAFLD patients receiving liver biopsy showed that progressive NASH or advanced fibrosis was the actual cause of long-term increased cardiovascular events [22–24]. Similar results were derived from a large cohort study in 229 NAFLD patients who received liver biopsy and were followed up for 33 years. In this cohort study, the worst prognosis was seen in patients with advanced fibrosis. Furthermore, it stressed that advanced fibrosis was the strongest predictor for cardiovascular mortality in NAFLD patients, even better than NASH [25]. In the NHANES III study with a mean follow-up period of 14.5 years, NAFLD with advanced fibrosis assessed by NFS was an independent predictor of increased mortality, mainly from cardiovascular cause [10]. In another study of patients with a Framingham cardiovascular risk score ≥20%, the presence of advanced fibrosis was predictive of cardiovascular events [26]. Targher et al. [27] also demonstrated that NAFLD patients with greater severity of NAFLD fibrosis evaluated by liver biopsy had remarkably greater CIMT. Our results were consistent with these studies. However there is still no well-agreed explanation of the mechanism for the effect of NAFLD fibrosis on atherosclerosis. Pronounced insulin resistance in the progression of liver fibrosis may partly account for the association between severity of NAFLD fibrosis and atherosclerosis [5]. Considering the association still persisted after adjustment for HOMA_IR, additional factors may also play roles in the development of atherosclerosis in NAFLD fibrosis patients. Considerable experimental evidence justified a key role of transforming growth factor-β (TGF-β) in advanced fibrosis [28]. TGF-β is a major pro-fibrotic cytokine and up-regulates α-smooth muscle actin and type Icollagen produced by hepatic stellate cell-derived myofibroblasts [29]. Some study showed that TGF-β increase in adventitial collagen could lead to the progression of arterial stiffening [30]. Definitely, it still requires further studies to verify.

To the best of our knowledge, this is the first community-based study to explore the association between advance fibrosis assessed by NFS and subclinical atherosclerosis marked with elevated CIMT, presence of carotid plaque and elevated baPWV in NAFLD patients. We do acknowledge limitations of this study. Firstly, the cross-sectional nature of this study cannot elucidate the causative relationship. However, previous longitudinal studies were inclined to regard advanced fibrosis as the cause [10,22]. Secondly, although NFS was originally developed in relatively narrow NAFLD patients, it has been validated in other 13 studies with a total of more than 3000 patients in different ethnicities. Though this score is already applied in clinical practice, the included variables of BMI, age and diabetes in themselves are risk factors of atherosclerosis. This consideration highlights the necessity and importance of appropriate adjustment for these relevant variables. Thirdly, the positive association was only discovered in middle-aged and older Chinese population, which cannot be generalized to other ethnics and adolescent.

In conclusion, advanced fibrosis confirmed by NFS was positively associated with CIMT, carotid plaque and arterial stiffness in NAFLD patients, independent of conventional CVD risk factors and insulin resistance. For NAFLD patients detected by ultrasonography, NFS might be useful to assess future CVD risk, and early prevention

should be valued in NAFLD patients with NFS > 0.676.

Conflicts of interest

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.atherosclerosis.2015.05.002.

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