Predictive Value of Plasma PCSK9 Levels for Degree of Atherosclerosis and Major Adverse Cardiovascular and Cerebrovascular Events in Older Adult Patients with Non-Alcoholic Fatty Liver Disease

Jinrui Ji[1,2,*], Xiaoyun Wei[1,2,*], Dongyun Wan[1,2,*], Lei Wu[1,2,*], Guangyao Chen[1,3,*], Hengliang Liu[1,2,*]

[1]Clinical Medical Department, Faculty of Medicine, Henan University of Traditional Chinese Medicine, Zhengzhou, 450000, People's Republic of China; [2]Department of Cardiology, People's Hospital of Henan University of Traditional Chinese Medicine, Zhengzhou, 450000, People's Republic of China; [3]Zhengzhou Institute of Gastroenterology, People's Hospital of Henan University of Traditional Chinese Medicine, Zhengzhou, 450000, People's Republic of China

*These authors contributed equally to this work

Correspondence: Hengliang Liu, Henan University of Traditional Chinese Medicine, No. 33, Huanghe Road, Jinshui District, Zhengzhou, Henan Province, 450000, People's Republic of China, Tel/Fax +86 0371-67077158, Email hnzzjjr@163.com

Purpose: This study investigated the influence of plasma proprotein convertase subtilisin/kexin 9 (PCSK9) levels on the degree of atherosclerosis and major adverse cardiovascular and cerebrovascular events (MACCE) in older adults with non-alcoholic fatty liver disease.

Methods: The degree of atherosclerosis severity was assessed by the standard Gensini score quartile method. According to the degree of atherosclerosis, patients were divided into mild (0–24 points; n=84), moderate (25–53 points; n=86), and severe groups (≥54 points; n=84) and then categorized as MACCE (n=30) or non-MACCE (n=224) according to 6-month follow-up data. The patients' age, sex, smoking history, medical history, and early morning fasting venous blood, for measuring biochemical indexes, were collected. Clinical data were compared between groups and the relationship between Gensini scores and PCSK9 was evaluated.

Results: Compared with the mild group, the moderate and severe groups had higher high-sensitivity C-reactive protein(hs-CRP), PCSK9, triglycerides(TG), low-density lipoprotein cholesterol (LDL-C), and lipoprotein(a)[Lp(a)] levels and lower high-density lipoprotein cholesterol(HDL-C) levels (all P<0.05). Moreover, PCSK9 positively correlated with Gensini scores (r=0.657, P<0.01). The MACCE and non-MACCE groups had significantly different ages, statin use, Gensini scores, PCSK9, and LDL-C (all P<0.05). Multi-factorial Cox risk regression analysis showed the Gensini score (HR=1.018, 95% CI: 1.006~1.029) and PCSK9 (HR=1.147, 95% CI: 1.038~1.287) were independent risk factors for MACCE.

Conclusion: The Gensini score and PCSK9 levels can be used as predictive indicators for the degree of illness and occurrence of MACCE in older NAFLD patients.

Keywords: older adults, non-alcoholic fatty liver disease, proprotein convertase subtilisin/kexin 9, atherosclerosis, major adverse cardiovascular and cerebrovascular events

Introduction

Between population aging, the obesity epidemic, and the effective control of viral hepatitis, non-alcoholic fatty liver disease (NAFLD) has become the most common chronic liver disease worldwide, with increasing clinical complications, economic burden, and impact on the quality and longevity of patients' lives.[1,2] NAFLD is intrinsically linked to metabolic and cardiovascular disease and may be involved in cardiovascular disease pathogenesis by inducing insulin resistance, atherogenic dyslipidemia, and the release of systemic inflammatory mediators, coagulation mediators, and oxidative stressors.[3–5] Major adverse cardiovascular and cerebrovascular events (MACCE) are the leading cause of death

in patients with NAFLD,[6,7] especially in older NAFLD patients. The risk of cardiovascular events in this patient subgroup needs to be regularly assessed, even when their body mass index is normal. Detecting MACCE in older NAFLD patients at an early stage and intervening as early as possible is the key to improving their quality of life.[8]

Plasma proprotein convertase subtilisin/kexin 9 (PCSK9) is a member of the proprotein convertase family, and its functional changes are involved in the progression of cardiovascular disease and atherosclerosis.[9] Studies have found that higher serum PCSK9 levels are associated with higher Gensini coronary artery lesion scores, more severe atherosclerosis, and a greater risk of MACCE.[10,11] To our knowledge, the relationship between PCSK9 levels and the risk of MACCE in older NAFLD patients is unclear. In this study, we investigated the relationship between serum PCSK9 levels and the degree of atherosclerosis in patients with NAFLD and the predictive value of PCSK9 for the risk of developing MACCE.

Materials and Methods
Study Design and Patients
This study was approved by the Medical Ethics Committee of our hospital (No.20200129) and adopted a retrospective cohort study design (Figure 1). This study analyzed the medical records of 542 NAFLD patients who attended the People's Hospital of Henan University of Traditional Chinese Medicine from January 2020 to December 2022. We included (1) patients who met the American association for the study of liver diseases (AASLD) diagnosis of NAFLD.[12] (2) Patients aged≥18 years. (3) Patients with complete medical records.(4) Patients that underwent fasting abdominal color Doppler ultrasonography. (5) Patient who completes Gensini score after coronary angiography.

The exclusion criteria were (1) specific diseases causing fatty liver (eg, viral hepatitis, drug-induced liver disease, total parenteral nutrition, hepatomegaly, autoimmune liver disease; (2) combined dysfunction of the liver and other organs, such as the kidneys; (3) advanced malignancy, severe trauma, or systemic infection; (4) recent treatment with corticosteroids; and (5) missing clinical data.udy design and patients.

Data Collection
The patients' data were gathered from the hospital's patient case system (Winning Health Technology Group Co., Ltd., Shanghai) and included demographic information (age and sex),medical history (hypertension, diabetes, smoking, and statin use) and laboratory results{Triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), lipoprotein(a) (Lp(a)),high-sensitivity C-reactive protein (hs-CRP), fasting blood glucose (FBG),PCSK9,aspartate transaminase (AST) and alanine transaminase (ALT)]for 172 men and 82 women aged 68.2±5.1 years. There were 229 cases of multibranch lesions.

Fasting venous blood was collected within 24 hours of admission, and a fully automated biochemical instrument (HITACHI 7080, Hitachi Ltd, Tokyo, Japan) was used for measurement: TG,TC, HDL-C), LDL-C,LP(a), hs-CRP were determined by latex immunoturbidimetric method (catalog number 6840-IVD381,6840-IVD383,6840-IVD384,6840-IVD379,6840-IVD385,6840-IVD265), FBG were determined by hexokinase assay (catalog number 6840-IVD306), PCSK9,AST and ALT were determined by enzyme-linked immunosorbent assay (ELISA)(catalog number 6840-IVD255,6840-IVD344,6840-IVD343)

Coronary angiography was performed by interventional cardiologists using a GE Innova 3100 digital subtraction angiography machine (General Electric [GE] Company, Boston, USA). Appropriate stent implantation was selected according to the intraoperative lesions, with final angiographic confirmation of Thrombolysis in Myocardial Infarction flow up to grade 3.

All patients were diagnosed with fatty liver based on the ultrasonographic findings. All patients underwent fasting abdominal color Doppler ultrasonography (GE730, GE Company), which was consistent with the following imaging manifestations:[13] (1) diffuse enhancement of near-field echoes in the liver region was stronger than that of the kidneys and spleen, with gradual attenuation of far-field echoes; (2) intrahepatic ductal structures were poorly visualized; (3) the liver was mildly to moderately enlarged with rounded edge angles; (4) color Doppler flow imaging showed that the intrahepatic color flow signal was reduced or difficult to visualize, but the intrahepatic vascular course was normal; and
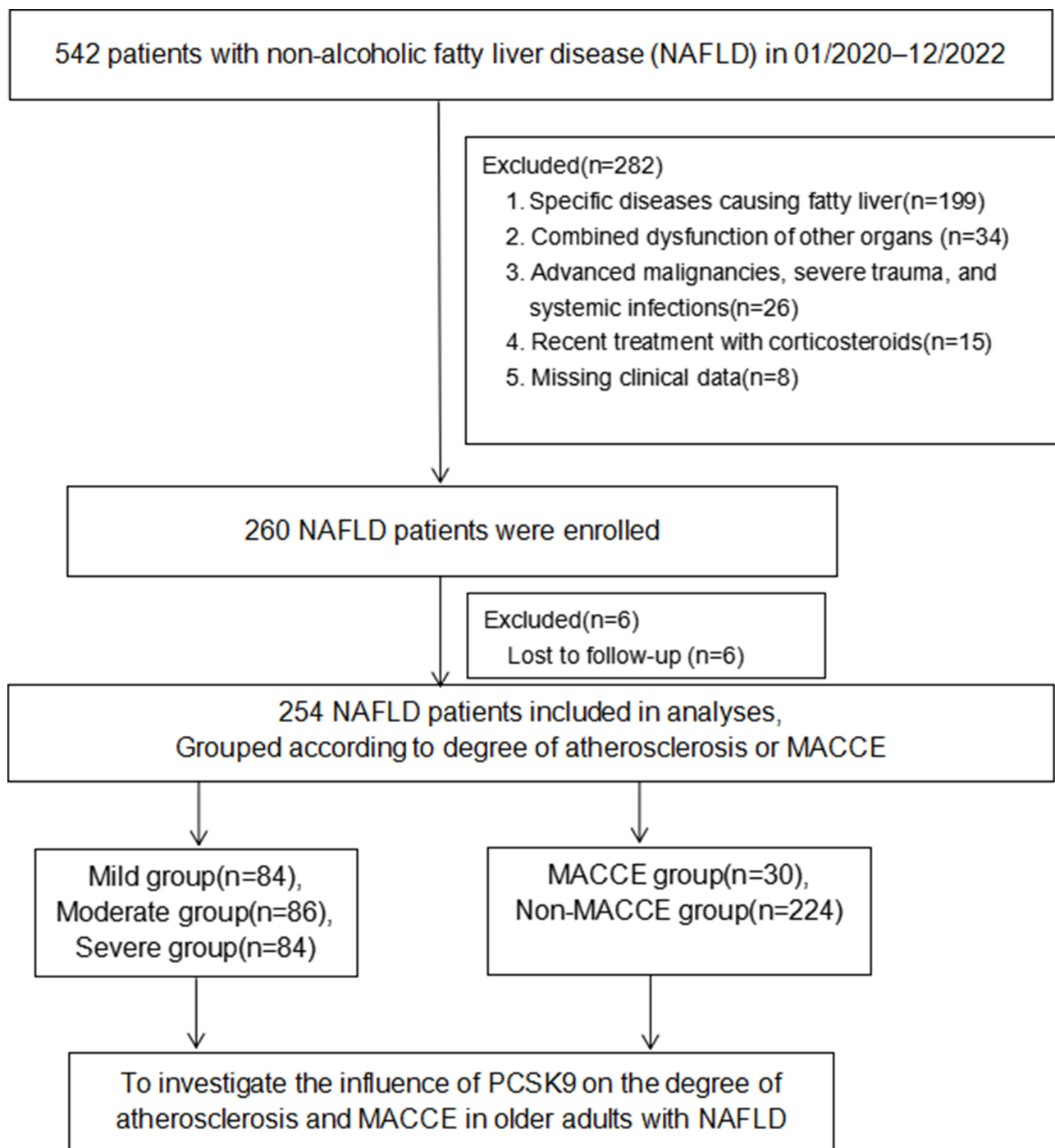
Figure 1 Flowchart of the study.

(5) the right lobe of the liver peritoneum and diaphragmatic echoes were not clear or incomplete. Color Doppler ultrasonography was performed by physicians with more than 5 years of experience.

We used the following definitions for the patients' characteristics. Smoking: history of smoking≥1/d for >6 months; hypertension: use of antihypertensive medication, or blood pressure 140/90 mmHg (1 mmHg=0.133 kPa); diabetes:[14] random blood glucose ≥11.1 mmol/L or FBG ≥7.0 mmol/L and 2-h postprandial blood glucose ≥11.1 mmol/L; multivessel disease: presence of at least two stenoses located in two branches of the major subepicardial coronary arteries (left anterior descending, right coronary, left circumflex) or one subepicardial coronary artery and one branch

of another coronary artery >2.5 mm in diameter with >50% stenosis. MACCE included all-cause death (cardiac and noncardiac death), cardiac death (death from malignant arrhythmias, heart failure, and myocardial infarction), myocardial infarction (diagnosed by hospitalization for signs and symptoms, including nonfatal myocardial infarction and myocardial infarction-related death), stroke (ischemic or nonischemic stroke-related hospitalizations or deaths), revascularization (revascularization procedures performed for any lesion, including coronary artery bypass grafting and percutaneous coronary intervention), and in-stent thrombosis as defined by the American Academic Research Consortium.[15]

Calculation of Gensini Score

The degree of atherosclerosis in patients with NAFLD was assessed using the standard Gensini score quartile method. The Gensini score is the product of the coronary lumen diameter stenosis score and the lesion site coefficient.[16] The Gensini scores and their corresponding coronary artery lumen diameter stenosis rates are as follows: 1, 1%–25%; 2, 26%–50%; 4, 51%–75%; 8, 76%–90%; 16, 91%–99%; and 32, total occlusion.

Outcome Measures

According to the Gensini score, the patients were divided into the mild group (0–24 points), moderate group (25–53 points), and severe group (≥54 points). They were further divided into two groups(MACCE group and Non-MACCE group) according to the occurrence of MACCE at the 6-month post-discharge follow-up. In this study, we investigated the relationship between serum PCSK9 levels and the degree of atherosclerosis in patients with NAFLD and the predictive value of PCSK9 for the risk of developing MACCE.

Statistical Analysis

SPSS 25.0 statistical software was used to process the data. Count data were expressed as the number (%) of cases. The χ^2 test was performed for comparison between groups, and the rank-sum test was used for rank data between multiple groups. Continuous variables with a normal distribution were expressed as the mean±standard deviation. The One-way ANOVA was performed for comparisons among multiple groups, and the independent samples *t*-test was performed for comparisons between two groups. Non-normally distributed continuous variables were described as medians (interquartile ranges) and assessed using Kruskal–Wallis test (three groups).Pearson's analysis was used to analyze the relationship between the Gensini score and PCSK9.Statistically significant independent variables were subjected to one-way Cox modeling with a significance test level of 0.05, and then multifactorial Cox modeling was performed using the stepwise method. Receiver operating characteristic curves were used to analyze the predictive value of PCSK9 for the occurrence of MACCE in NAFLD patients.The area under the curve(AUC) values were compared using the DeLong test. Results with P-values less than 0.05 were considered statistically significant.

Results

Gensini Score Subgroup Clinical Data and Correlation Analysis

Compared with the mild group, the moderate and severe groups had significantly higher hs-CRP, PCSK9, TG, LDL-C, and Lp(a) and significantly lower HDL-C; the differences between the groups were statistically significant (P<0.05 for all comparisons). The differences in age, sex, hypertension, diabetes, smoking history, statin medication, TC, FBG, AST, and ALT indicators were not statistically significant (P>0.05 for all comparisons, Table 1). Pearson correlation analysis showed that PCSK9 was positively correlated with the Gensini score (r=0.657, P<0.01).

Comparison of clinical data between MACCE and non-MACCE groups

Compared with the non-MACCE group, patients in the MACCE group were significantly older and had significantly higher Gensini scores, PCSK9, and LDL-C and significantly lower statin use (all P<0.05). The differences in other indicators were not statistically significant (P>0.05, Table 2).

Table 1 Clinical Data Analysis for Gensini Score Subgroups

Variable	Mild group, n=84	Moderate group, n=86	Severe group, n=84	F/χ^2/Z	P
Age (years)	68.4±5.4	68.1±5.0	69.1±5.5	0.376	0.740
Male sex	57 (67.86)	56 (65.12)	59 (70.24)	0.511	0.775
Hypertension	27 (32.14)	31 (36.05)	34 (40.48)	1.264	0.531
Diabetes	16 (19.05)	21 (24.42)	17 (20.24)	0.811	0.667
History of smoking	42 (50.00)	38 (44.19)	40 (47.62)	0.583	0.747
Statins	46 (54.76)	50 (58.14)	53 (63.10)	1.217	0.544
Hs-CRP [mg/L, M(P$_{25}$, P$_{75}$)]	5.79, (4.17, 7.81)	6.22 (4.61, 7.58)[a]	7.85 (6.21, 9.11)[a,b]	37.304	<0.01
PCSK9 (ng/mL)	329.17±50.26	416.33±55.92[a]	498.55±61.28[a,b]	135.202	<0.01
TG (mmol/L)	1.47±0.33	1.65±0.36[a]	1.86±0.24[a,b]	11.394	<0.01
TC (mmol/L)	4.99±0.35	4.15±0.63	4.17±0.85	1.305	0.196
LDL-C (mmol/L)	2.69±0.52	2.87±0.35[a]	3.95±0.48[a,b]	53.392	<0.01
HDL-C (mmol/L)	1.46±0.25	1.20±0.22[a]	1.06±0.27[a,b]	22.793	<0.01
Lp(a) (mg/L)	0.52±0.12	0.63±0.09[a]	0.70±0.11[a,b]	10.542	<0.01
FBG (mmol/L)	5.97±1.63	5.98±1.69	5.99±1.72	0.063	0.962
AST (U/L)	23.23±4.58	23.29±4.60	24.59±4.95	0.058	0.950
ALT (U/L)	22.55±4.11	22.58±5.29	22.61±5.54	0.639	0.719

Note: a Comparison with mild group; b comparison with moderate group. Data are presented as mean±standard deviation or number (%) unless otherwise specified.
Abbreviations: hs-CRP, high-sensitivity C-reactive protein; PCSK9, plasma proprotein convertase subtilisin/kexin 9; TG, triglycerides; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; Lp(a), lipoprotein(a); FBG, fasting blood glucose; AST, aspartate transaminase; ALT, alanine transaminase.

Table 2 Comparison of Clinical Data Between MACCE and Non-MACCE Groups

Variable	MACCE group n=30	Non-MACCE group n=224	t/χ^2	P
Age (years)	72.1±5.5	68.6±3.4	3.365	0.001
Male sex	21 (70.00)	151 (67.41)	0.081	0.776
Hypertension	14 (46.67)	90 (40.18)	0.461	0.497
Diabetes	6 (20.00)	27 (12.05)	0.859	0.354
History of smoking	14 (46.67)	83 (37.05)	1.036	0.309
Statins	14 (46.67)	170 (75.89)	37.304	0.001
Multivessel disease	28 (93.33)	201 (89.73)	0.087	0.768
Gensini score	88.10±26.70	44.08±23.31	9.902	<0.01
PCSK9 (ng/mL)	406.5±81.8	310.5±49.6	9.101	<0.01
TG (mmol/L)	1.39±0.48	1.50±0.51	1.117	0.265
TC (mmol/L)	4.16±0.84	4.39±0.80	1.470	0.143
LDL-C (mmol/L)	2.88±0.79	2.52±0.77	2.398	0.017
HDL-C (mmol/L)	1.36±0.25	1.37±0.22	0.230	0.818
Lp(a) (mg/L)	0.53±0.13	0.55±0.10	0.990	0.323
FBG (mmol/L)	5.89±1.65	5.95±1.62	0.190	0.849
AST (U/L)	23.35±4.39	23.27±4.42	0.093	0.926
ALT (U/L)	22.57±4.64	22.59±4.63	0.022	0.982

Note: Data are presented as mean±standard deviation or number (%).
Abbreviations: MACCE, major adverse cardiovascular and cerebrovascular events;hs-CRP, high-sensitivity C-reactive protein; PCSK9, plasma proprotein convertase subtilisin/kexin 9; TG, triglycerides; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; Lp(a), lipoprotein(a); FBG, fasting blood glucose; AST, aspartate transaminase; ALT, alanine transaminase.

Cox Analysis of the Risk of MACCE

One-way and multi-factorial Cox risk regression analyses were performed with MACCE as the dependent variable and age, Gensini score, hs-CRP, PCSK9, TG, LDL-C, HDL-C, and Lp(a) as the independent variables. The results showed that the Gensini score and PCSK9 were independent risk factors for the occurrence of MACCE (P<0.05, Table 3 and Table 4,Figures 2 and 3).

The value of the Gensini score and PCSK9, alone and combined, to predict MACCE

The area under the curve (AUC) for Gensini score and PCSK9 to predict MACCE were 0.896 and 0.839, respectively. The sensitivities were 84.38% and 75.89% and the specificities were 80.00% and 80.00% at a Gensini score of 67 and PCSK9 of 344.7 ng/mL, respectively. The predictive value of the two tests combined was significantly increased: the AUC was 0.942, the difference was statistically significant(Z=2.845,P=0.004;Z=4.376,P<0.001), the sensitivity was 89.29%, and the specificity was 90.00% (Figure 4).

Discussion

According to the latest research data, the global prevalence of NAFLD in adults is as high as 32.4%.[17–19] The incidence rate in developed countries is stabilizing, but the rate in developing countries is increasing significantly. In 2020, Eslam

Table 3 Variable Assignment Table

Variable	Variable assignment
Dependent variable	
MACE	Yes=1; No=0
Independent variable	
Age (years)	<75=1; ≥75=0
Gensini score	<49=1; ≥49=0
hs-CRP(mg/L)	<321.8=1; ≥321.8=0
PCSK9 (ng/mL)	<6.63=1; ≥6.63=0
TG(mmol/L)	<1.70=1; ≥1.70=0
LDL-C(mmol/L)	<3.40=1; ≥3.40=0
HDL-C(mmol/L)	<1.00=1; ≥1.00=0
Lp(a) (mg/L)	<0.5=1; ≥0.5=0

Abbreviations: MACCE, major adverse cardiovascular and cerebrovascular events;hs-CRP, high-sensitivity C-reactive protein; PCSK9, plasma proprotein convertase subtilisin/kexin 9; TG, triglycerides; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; Lp(a), lipoprotein(a).
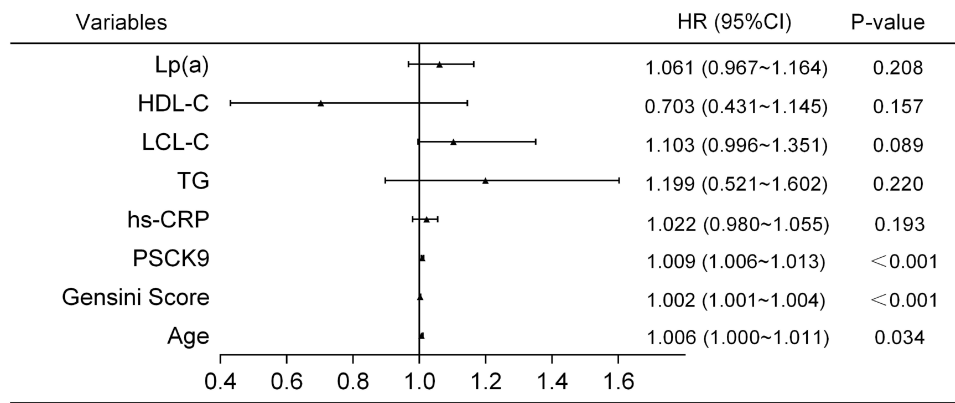
Table 4 One-Way and Multi-Factorial Cox Analyses of MACCE

Variables	One-way Cox		Multi-factorial Cox	
	OR(95% CI)	P	OR(95% CI)	P
Age	1.006(1.000~1.011)	0.034	1.061(0.967~1.164)	0.281
Gensini Score	1.002(1.001~1.004)	<0.001	1.002(1.000~1.004)	0.029
PSCK9	1.009(1.006~1.013)	<0.001	1.008(1.004~1.012)	<0.001
hs-CRP	1.022(0.980~1.055)	0.193	-	-
TG	1.199(0.521~1.602)	0.220	-	-
LCL-C	1.103(0.996~1.351)	0.089	-	-
HDL-C	0.703(0.431~1.145)	0.157	-	-
Lp(a)	1.061(0.967~1.164)	0.208	-	-

Abbreviations: hs-CRP, high-sensitivity C-reactive protein; PCSK9, plasma proprotein convertase subtilisin/kexin 9; TG, triglycerides; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; Lp(a), lipoprotein(a).
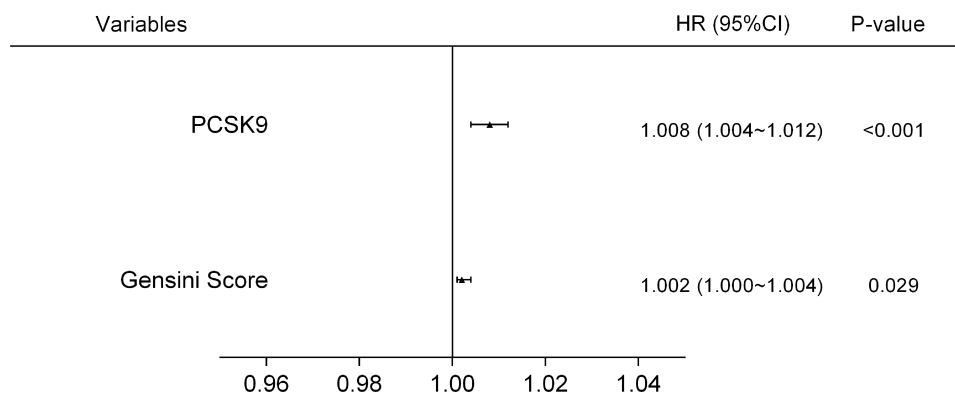
Variables		HR (95%CI)	P-value
Lp(a)		1.061 (0.967~1.164)	0.208
HDL-C		0.703 (0.431~1.145)	0.157
LCL-C		1.103 (0.996~1.351)	0.089
TG		1.199 (0.521~1.602)	0.220
hs-CRP		1.022 (0.980~1.055)	0.193
PSCK9		1.009 (1.006~1.013)	<0.001
Gensini Score		1.002 (1.001~1.004)	<0.001
Age		1.006 (1.000~1.011)	0.034

Figure 2 One-way Cox analysis.
Abbreviations: hs-CRP, high-sensitivity C-reactive protein; PCSK9, plasma proprotein convertase subtilisin/kexin 9; TG, triglycerides; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; Lp(a), lipoprotein(a).

Variables		HR (95%CI)	P-value
PCSK9		1.008 (1.004~1.012)	<0.001
Gensini Score		1.002 (1.000~1.004)	0.029

Figure 3 Multi-factorial Cox analysis.
Abbreviations: PCSK9, plasma proprotein convertase subtilisin/kexin 9.
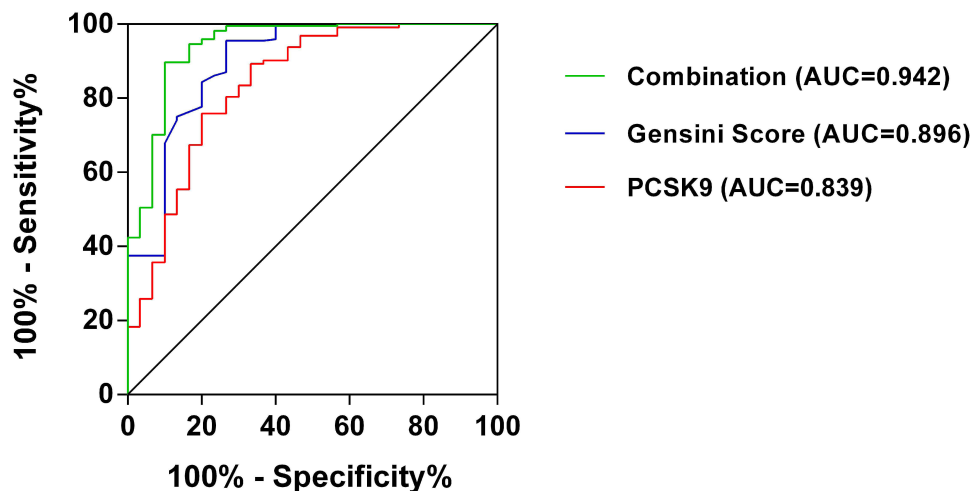

Figure 4 Receiver operating characteristics curve.

et al suggested that NAFLD be renamed metabolism-associated fatty liver disease to clarify that it is an acquired metabolic stress–related liver injury.[20] The disease is closely related to insulin resistance and genetic susceptibility and is further associated with a high prevalence of metabolic diseases, such as diabetes mellitus, which is often accompanied by

other metabolic disorders and multisystemic diseases. Therefore, fatty liver should be valued as an early warning sign of cardiovascular disease.[21]

PCSK9 is a serine protease mainly expressed in the liver and can bind to the LDL receptor (LDLR) on the surface of the cell membrane to form a complex. PCSK9 downregulates the density of the receptor, which reduces its effect on LDL clearance and increases the level of LDL in circulation. PCSK9 is also involved in the regulation of inflammation in the body.[22] Studies have shown that PCSK9 plays a pro-inflammatory role in the development of atherosclerosis by activating the TLR4/NF-κB signaling pathway and increasing the expression of pro-inflammatory factors, and the body's resulting pro-/anti-inflammatory imbalance may further contribute to the onset and progression of atherosclerosis.[23] Cohen et al found that PSCK9 loss-of-function mutations can reduce serum LDL-C levels by 15%~28%. They further found that ischemic heart disease was associated with a significant increase in serum LDL-C levels of 28% and reduced the incidence of ischemic heart disease by 47%–88%.[24] In this study, patients with NAFLD combined with coronary heart disease were selected as research subjects and grouped according to their Gensini scores. The results showed that hs-CRP, PCSK9, TG, LDL-C, and Lp(a) were significantly higher in the severe group than in the moderate and mild groups. The positive correlation between Gensini scores and PCSK9 suggests that serum PCSK9 can be used as a blood marker for the degree of atherosclerosis in older NAFLD patients. The results of a randomized, double-blind, controlled clinical trial by Raal et al showed that treatment with the monoclonal antibody against PCSK9, evolocumab, could reduce LDL-C levels.[25] Sabatine et al conducted a non-blinded, randomized clinical trial in which 4465 patients were divided into two groups and treated with the standard regimen alone or combined with evolocumab,[26] The results showed that LDL-C levels were lower in the group treated with both evolocumab and the standard regimen than in the group that received the standard regimen alone (P<0.001), suggesting that inhibition of PCSK9 levels in the blood can reduce LDL-C levels.These results are consistent with our previous findings.[27] Based on our previous study, we found that PCSK9 modulates LDL-C to alter the risk of cardiovascular occurrence, suggesting that PCSK9 can be used as a predictor of the risk of MACCE in older adult NAFLD patients.

Huang J et al found that Serum PCSK9 levels were positively correlated with multi-vessel CHD and Gensini score.[28] Recently, a regression study showed that inhibition of PCSK9 can lead to atheroma regression and plaque stabilization in coronary arteries, reducing cardiovascular events.[29]

Furthermore, PCSK9 levels greater than 310 ng/mL were associated with more severe coronary artery stenosis and a higher incidence of MACCE at follow-up.[30] The difference in traditional atherosclerosis risk factors in different Gensini score subgroups was not statistically significant in this study, possibly because the selected patients were older adults. Theocharidou et al reported that PCSK9 was associated with higher morbidity, mortality, and risk of major clinical adverse events in patients with NAFLD.[31] Elevated PCSK9 is common in patients with acute myocardial infarction and heart failure, and the risk of developing MACCE and heart failure is higher with PCSK9 compared with LDL-C.[32] In the SPIRE-1 and SPIRE-2 Phase III clinical trials, 27,438 patients were enrolled and randomized into a control group or an experimental group that received subcutaneous injections of a monoclonal antibody against PCSK9 (bococizumab, 150 mg once every 2 weeks).[33] In patients with low cardiovascular risk, the difference in the incidence rate of major adverse cardiovascular events between the control group and the test group was not statistically significant. However, in patients with high cardiovascular risk, the incidence rate of major adverse cardiovascular events was lower in the test group than in the control group. The rates of all adverse events were lower in the test group than in the control group. In the phase III clinical trial of evolocumab, compared with the placebo group, patients with cardiovascular disease in the evolocumab group had a 59% reduction in LDL-C from baseline at week 48, and cardiogenic death, myocardial infarction, stroke, and death due to hospitalization for unstable angina or coronary revascularization were reduced from 11.3% to 9.8% (P<0.001).[22] The results of these two studies suggest that reducing circulating levels of PCSK9 reduces the risk of MACCE. The AUC, sensitivity, and specificity of PCSK9 in this study were slightly worse than those of the Gensini score, which may be related to the fact that PCSK9 is affected by a variety of factors, such as abnormal lipid metabolism. However, the AUC of the two tests combined was significantly higher than that of the single indicator test (0.942, with a sensitivity of 89.29% and a specificity of 90.00%), suggesting that the incidence of MACCE in older NAFLD patients can be assessed by combining the Gensini score and PCSK9 levels before atherosclerosis treatment.

Limitations

The present study was a single-center study and included a small number of patient cases, which may have led to biased results. This study ignored the effect of drug use factors such as hypertension and diabetes.These limitations may affect the conclusions that can be drawn from this study. Validating these conclusions in multicenter studies with larger sample sizes and additional observational indices will enable more accurate assessments of the diagnostic efficacy of PCSK9 in the risk of MACCE in older patients with NAFLD.

Conclusion

Serum PCSK9 is associated with independent risk factors for MACCE in older adults with NAFLD. The Gensini score and PCSK9 levels can be used as predictive indicators for the degree of illness and occurrence of MACCE in older NAFLD patients.

Data Sharing Statement

Further inquiries can be acquired directly to the corresponding author.

Ethics Approval

This study was approved by the Medical Ethics Committee of The Fifth Clinical Medical College of Henan University of Traditional Chinese Medicine (No.20200129).As this study is retrospective and presents no risk of harm to subjects, and no privacy of individuals is exposed, informed consent was waived. All procedures adhered to the ethical standards and the Helsinki Declaration.

Acknowledgments

Disclosure

The authors report no conflicts of interest in this work.

References

1. Huang DQ, El-Serag HB, Loomba R. Global epidemiology of NAFLD-related HCC: trends, predictions, risk factors and prevention. *Nat Rev Gastroenterol Hepatol*. 2021;18(4):223–238. doi:10.1038/s41575-020-00381-6
2. Younossi ZM, Koenig AB, Abdelatif D, et al. 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
3. Adams LA, Anstee QM, Tilg H, et al. Non-alcoholic fatty liver disease and its relationship with cardiovascular disease and other extrahepatic diseases. *Gut*. 2017;66(6):1138–1153. doi:10.1136/gutjnl-2017-313884
4. Dewidar B, Kahl S, Pafili K, Roden M. Metabolic liver disease in diabetes - From mechanisms to clinical trials. *Metabolism*. 2020;111:154299. doi:10.1016/j.metabol.2020.154299
5. Gutiérrez-Cuevas J, Santos A, Armendariz-Borunda J. Pathophysiological molecular mechanisms of obesity: a link between MAFLD and NASH with cardiovascular diseases. *Int J Mol Sci*. 2021;22(21):11629. doi:10.3390/ijms222111629
6. Chalasani N, Younossi Z, Lavine JE. Lavine JE,et al. the diagnosis and management of nonalcoholic fatty liver disease: practice guidance from the American association for the study of liver diseases. *Hepatology*. 2018;67(1):328–357. doi:10.1002/hep.29367
7. Loria P, Adinolfi LE, Bellentani S, et al. Practice guidelines for the diagnosis and management of nonalcoholic fatty liver disease. A decalogue from the Italian association for the study of the liver (AISF) expert committee. *Dig Liver Dis*. 2010;42(4):272–282. doi:10.1016/j.dld.2010.01.021
8. Simon TG, Roelstraete B, Hagström H, et al. Non-alcoholic fatty liver disease and incident major adverse cardiovascular events: results from a nationwide histology cohort. *Gut*. 2022;71(9):1867–1875. doi:10.1136/gutjnl-2021-325724
9. Barale C, Melchionda E, Morotti A, Russo I. PCSK9 biology and its role in atherothrombosis. *Int J Mol Sci*. 2021;22(11):5880. doi:10.3390/ijms22115880
10. Gao J, Yang YN, Cui Z, et al. Pcsk9 is associated with severity of coronary artery lesions in male patients with premature myocardial infarction. *Lipids Health Dis*. 2021;20(1):56. doi:10.1186/s12944-021-01478-w
11. Dalgic Y, Abaci O, Kocas C, et al. The relationship between protein convertase subtilisin kexin type-9 levels and extent of coronary artery disease in patients with non-ST-elevation myocardial infarction. *Coron Artery Dis*. 2020;31(1):81–86. doi:10.1097/MCA.0000000000000774
12. Fan JG, Jia JD, Li YM, et al. Guidelines for the diagnosis and management of nonalcoholic fatty liver disease: update 2010: (published in Chinese on Chinese. *J Hepatol*. 2010;18:163–166).
13. Saadeh S, Younossi ZM, Remer EM, et al. The utility of radiological imaging in nonalcoholic fatty liver disease. *Gastroenterology*. 2002;123 (3):745–750. doi:10.1053/gast.2002.35354

14. ElSayed NA, Aleppo G, Aroda VR, et al. Summary of revisions: standards of care in diabetes-2023. *Diabetes Care*. 2023;46:S5–S9.

15. Cutlip DE, Windecker S, Mehran R, et al. clinical end points in coronary stent trials: a case for standardized definitions. *Circulation*. 2007;115 (17):2344–2351. doi:10.1161/CIRCULATIONAHA.106.685313

16. Rampidis GP, Benetos G, Benz DC, et al. A guide for gensini score calculation. *Atherosclerosis*. 2019;287:181–183. doi:10.1016/j. atherosclerosis.2019.05.012

17. Friedman SL, Neuschwander-Tetri BA, Rinella M, et al. Mechanisms of NAFLD development and therapeutic strategies. *Nat Med*. 2018;24 (7):908–922. doi:10.1038/s41591-018-0104-9

18. Riazi K, Azhari H, Charette JH, et al. The prevalence and incidence of NAFLD worldwide: a systematic review and meta-analysis. *Lancet Gastroenterol Hepatol*. 2022;7(9):851–861. doi:10.1016/S2468-1253(22)00165-0

19. Younossi Z, Tacke F, Arrese M, et al. Global perspectives on nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. *Hepatology*. 2019;69 (6):2672–2682. doi:10.1002/hep.30251

20. Eslam M, Newsome PN, Sarin SK, 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

21. Rinella ME, Neuschwander-Tetri BA, Siddiqui MS, et al. AASLD practice guidance on the clinical assessment and management of nonalcoholic fatty liver disease. *Hepatology*. 2023;77(5):1797–1835. doi:10.1097/HEP.0000000000000323

22. Sabatine MS, Giugliano RP, Keech AC, et al. Evolocumab and clinical outcomes in patients with cardiovascular disease. *N Engl J Med*. 2017;376 (18):1713–1722. doi:10.1056/NEJMoa1615664

23. Tang ZH, Peng J, Ren Z, et al. New role of PCSK9 in atherosclerotic inflammation promotion involving the TLR4/NF-κB pathway. *Atherosclerosis*. 2017;262:113–122. doi:10.1016/j.atherosclerosis.2017.04.023

24. Cohen JC, Boerwinkle E, Mosley TH, et al. Sequence variations in PCSK9, low LDL, and protection against coronary heart disease. *N Engl J Med*. 2006;354(12):1264–1272. doi:10.1056/NEJMoa054013

25. Raal FJ, Honarpour N, Blom DJ, et al. Inhibition of PCSK9 with evolocumab in homozygous familial hypercholesterolaemia (TESLA Part B): a randomised, double-blind, placebo-controlled trial. *Lancet*. 2015;385(9965):341–350. doi:10.1016/S0140-6736(14)61374-X

26. Sabatine MS, Giugliano RP, Wiviott SD, et al. Efficacy and safety of evolocumab in reducing lipids and cardiovascular events. *N Engl J Med*. 2015;372(16):1500–1509. doi:10.1056/NEJMoa1500858

27. Ji J, Wei X, Chen W, et al. Effects of early PCSK9 inhibitor application on inflammation levels and microcirculatory function after PCI in patients with NSTE-ACS. *Am J Transl Res*. 2023;15(5):3586–3596.

28. Huang J, Gu JX, Wang K, et al. Association between serum PCSK9 and coronary heart disease in patients with type 2 diabetes mellitus. *Diabetol Metab Syndr*. 2023;15(1):260. doi:10.1186/s13098-023-01238-z

29. Biccirè FG, Häner J, Losdat S, et al. Concomitant coronary atheroma regression and stabilization in response to lipid-lowering therapy. *J Am Coll Cardiol*. 2023;82(18):1737–1747. doi:10.1016/j.jacc.2023.08.019

30. Santoso A, Yulianto Y, Simarmata H, et al. Effect of PCSK9 E670G and R46L polymorphisms on major adverse cardio-cerebrovascular events in patients with ST-segment elevation myocardial infarction undergoing primary percutaneous coronary intervention. *Int J Angiol*. 2021;30(01):22–28. doi:10.1055/s-0041-1722875

31. Theocharidou E, Papademetriou M, Reklou A, et al. The role of PCSK9 in the pathogenesis of non-alcoholic fatty liver disease and the effect of PCSK9 inhibitors. *Curr Pharm Des*. 2018;24(31):3654–3657. doi:10.2174/1381612824666181010123127

32. Zhang D, Mi Z, Peng J, et al. Nonalcoholic fatty liver disease as an emerging risk factor and potential intervention target for atherosclerotic cardiovascular diseases. *J Cardiovasc Pharmacol*. 2023;81(5):327–335. doi:10.1097/FJC.0000000000001418

33. Ridker PM, Revkin J, Amarenco P, et al. Cardiovascular efficacy and safety of bococizumab in high-risk patients. *N Engl J Med*. 2017;376 (16):1527–1539. doi:10.1056/NEJMoa1701488