European Review for Medical and Pharmacological Sciences 2024; 28: 1052-1059

The relationship and diagnostic efficacy of N-terminal propeptide type III collagen in pathological changes associated with non-alcoholic steatohepatitis

Y.-J. MI, Z. ZHANG, S.-L. JIN, H.-M. YAN, X.-M. YANG, J. ZHAO, R.-X. LIU, Y. WU

Clinical Laboratory Center, The First Hospital of Hebei Medical University, Shijiazhuang, China

Yujing Mi and Zheng Zhang are co-first authors

Abstract. – OBJECTIVE: This study delves into the role of N-terminal propeptide type III collagen (PIIINP) in the diagnosis and management of liver pathological changes associated with non-alcoholic steatohepatitis (NASH).

PATIENTS AND METHODS: We collected baseline information, pathological data, and serum PIIINP levels of 168 patients diagnosed with non-alcoholic fatty liver disease (NAFLD) *via* ultrasound imaging in our hospital. Based on the non-alcoholic fatty liver disease activity score (NAS), patients with different NAFLD patterns were divided into a Definite NASH group and a Not/borderline group. Differences in PIIINP levels and pathological features between the two groups were compared and analyzed. The diagnostic value of PIIINP for NASH was evaluated using the receiver operating characteristic (ROC) curve.

RESULTS: Patients with NASH exhibited significantly higher values of homeostatic model assessment for insulin resistance (HOMA-IR), fibrosis biomarker fibrosis-4 (FIB-4), aminotransferase-to-platelet ratio index (APRI), and serum PIIINP levels than those classified as Not/borderline. A marked increase in the serum concentrations of PIIINP was observed with the severity of fatty degeneration, lobular inflammation, and hepatocellular ballooning. The AUC of PIIINP for diagnosing definite NASH was 0.766 (95% CI: 0.694, 0.839), APRI was 0.634 (95% CI: 0.549, 0.718), and FIB-4 was 0.621 (95% CI: 0.534, 0.708). The AUC of PIIINP for diagnosing definite NASH was significantly higher than that of APRI and FIB-4 (all $p<0.05$). Utilizing the predetermined threshold values for diagnostic parameters, the PIIINP measure demonstrated a sensitivity of 71.6% and a specificity of 73.6% in diagnosing definitive NASH when its value exceeded 7.72 ng/dL. This yielded a Youden index of 0.45. Similarly, when the APRI measure exceeded 0.21, it exhibited a sensitivity of 60.5% and a specificity of 63.2%, resulting in a Youden index of 0.24. Moreover, when the FIB-4 index surpassed 0.26, it showed a sensitivity of 46.9% and a specificity of 79.3%, culminating in a Youden index of 0.26.

CONCLUSIONS: NASH patients in this study exhibited significantly elevated PIIINP serum levels, which were closely associated with hepatocyte pathological changes. PIIINP demonstrated superior competence in diagnosing NASH than APRI and FIB-4 and thus offers a viable alternative for the clinical diagnosis of NASH.

Key Words:

Non-alcoholic fatty liver disease, Non-alcoholic steatohepatitis, N-terminal propeptide type III collagen, Receiver operating characteristic curve.

Introduction

Non-alcoholic fatty liver disease (NAFLD) represents the hepatic manifestation of chronic metabolic syndrome and is predominantly caused by excessive accumulation of lipids in hepatic parenchyma (5-10% of the total liver volume). Besides, patients with such pathological alterations typically do not have a background of excessive alcohol consumption, viral infections, or exposure to toxins[1,2]. NAFLD is a disease spectrum that encompasses non-alcoholic hepatic steatosis, non-alcoholic steatohepatitis (NASH), cirrhosis, and hepatocellular carcinoma (HCC)[3]. With the growing prevalence of obesity and overweight individuals, NAFLD has emerged as an increasingly serious health issue, as its morbidity rate ranges from 6.3 to 45% among the adult population, with 10-30% of which are diagnosed as NASH[4]. The

Corresponding Author: Yan Wu, MD; e-mail: wycyhwy@163.com

etiological factors underpinning NAFLD include dietary habits, obesity, microbiome, lipid homeostasis imbalance, excessive lipid accumulation, and parenchymal inflammatory cell infiltration. With a disease spectrum from steatosis to NASH, NAFLD may eventually lead to irreversible liver damage, such as cirrhosis and HCC. Recent research[5] has substantiated the key role of the fibrosis stage in predicting liver disease outcomes. Therefore, the identification of late-stage NAFLD fibrosis stands as a pivotal factor in disease management.

Type III collagen is a major protein involved in extracellular matrix remodeling and can lead to fibrotic tissue accumulation in multiple organs, such as the liver or kidneys[6]. N-terminal propeptide type III collagen (PIIINP) is an N-terminal polypeptide produced by aminopeptidase cleavage of procollagen type III peptide (PIIIP) before its deposition outside the hepatocyte. In this process, PIIINP and PIIIP are in equimolar concentrations and enter the bloodstream[7]. Hence, fluctuations in serum PIIINP levels may predict type III collagen synthesis. PIIIP levels have been established as a marker of fibrosis in patients with liver disease, where the serum levels of 50 KD PIIINP progressively rise with the worsening of hepatic fibrosis, demonstrating a significant positive correlation with the severity of liver fibrosis[8]. Furthermore, research[9] also indicates that PIIINP is a non-invasive marker for diagnosing liver fibrosis in adults with chronic viral hepatitis. Nevertheless, its clinical value in NAFLD remains unknown.

Patients and Methods

Study Design

This study enrolled 168 patients who had either been diagnosed with severe hepatic steatosis through ultrasound imaging or had consistently elevated serum transaminase levels for 6 months or more. These patients voluntarily agreed to undergo a liver biopsy to determine the severity of NAFLD. Prior to inclusion, all patients were screened to rule out secondary causes of hepatic steatosis, including viral hepatitis, autoimmune hepatitis, Wilson's disease, α-1-antitrypsin deficiency, endocrine, genetic, and metabolic disorders, steatorrhea, alcohol consumption, and use of medications known to induce hepatic steatosis. Simultaneously, serum PIIINP levels were measured before liver biopsy, and histopathological changes in liver tissue were recorded after the biopsy. The study was approved by the First Hospital of Hebei Medical University Ethical approval No.: FHHMU-105214).

Inclusion and Exclusion Criteria

Inclusion criteria: (1) aged 18-65 years irrespective of gender; (2) qualitative liver ultrasonography showing fatty liver or MRI indicating a hepatic fat percentage above 10%; (3) liver biopsy results confirming NAFLD; (4) BMI of 25-40 kg/m^2; (5) presence of one metabolic risk factor (fasting blood glucose ≥7.0 mmol/L; or blood pressure ≥140/90 mmHg; cholesterol level >5.2 mmol/L; or fasting triglycerides >1.7 mmol/L).

Exclusion criteria: (1) history of other liver diseases (including hepatitis B or C virus infection, autoimmune liver disease, drug-induced liver injury, and metabolic liver disease); (2) history of excessive alcohol consumption (males: ≥163 g/day; females: ≥16 g/day)[10]; (3) presence of decompensated cirrhosis (ascites, gastrointestinal bleeding due to variceal rupture, liver failure, hepatic cellular failure, hepatorenal syndrome); (4) treatment with steatosis-inducing agents such as steroids, amiodarone, methotrexate, tamoxifen; (5) history of weight-loss surgery; (6) concurrent diseases that may cause PIIINP elevation, such as fractures, rheumatism, arthritis.

Liver Biopsy and Histological Typing

A liver biopsy was performed on all patients using a 16 G or 17 G biopsy needle under ultrasound guidance, and liver tissue samples in a length of 1.5 cm were collected. The collected liver tissues were pathologically analyzed by two experienced pathologists who were blind to patient information. The non-alcoholic fatty liver disease activity score (NAS)[11] was used to assess the histological features of NAFLD, encompassing steatosis (0-3 points), lobular inflammation (0-3 points), and hepatocyte ballooning (0-2 points). In the grading of hepatic steatosis, 0 signifies fatty degeneration involving less than 5% of hepatocytes, 1 corresponds to fatty degeneration involving up to 33% of hepatocytes, 2 represents fatty degeneration involving between 33% and 66% of hepatocytes, and 3 signifies fatty degeneration involving more than 66% of hepatocytes. In the grading of lobular inflammation, 0 denotes the absence of lesions, 1 corresponds to fewer than 2 lesions per 200x microscopic field, 2 represents fewer than 4 lesions per 200x microscopic field, and 3 signifies more than 4 lesions per 200x microscopic field. In terms of hepatocellular ballooning, 0 denotes

the absence of ballooned cells, 1 refers to a small number of ballooned cells, and 2 indicates many/prominent ballooned cells. The total NAS ranges from 0 to 8. Based on the NAS, participants were grouped into non-NASH (0-2 points), borderline NASH (3-4 points), and definite NASH (≥5 points).

Liver Function, Metabolic Indicators, and PIIINP Measurement

Fasting venous blood samples were collected after an 8-hour fast and centrifuged at 3,000 r/min for 10 min to obtain the serum, which was then aliquoted into EP centrifuge tubes and stored at -20°C for testing. The automated biochemical analyzer (Hitachi 70602, Guangdong, China) was used to measure serum liver enzyme indicators such as aspartate transaminase (AST), alanine transaminase (ALT), and γ-glutamyl transferase (GGT), lipid indicators such as total cholesterol (TC), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C), and triglycerides (TG), and fasting plasma glucose (FPG) and insulin levels. The homeostasis model assessment of insulin resistance (HOMA-IR) was used to estimate insulin resistance (IR), with HOMA-IR>2.5 considered as the threshold for IR. HOMA-IR, calculated as the product of fasting blood glucose (mmol/L) and fasting insulin (μU/mL) divided by 22.5, is typically considered normal when plasma insulin levels are around 5 μU/mL, corresponding to a blood glucose level of approximately 4.5 mmol/L in an ideal individual. HOMA-IR is a mathematical model established to reflect the interaction between glucose and insulin in different organs. This model only uses fasting blood glucose and fasting insulin values to evaluate the body's insulin resistance. The fibrosis index based on the 4 factor (FIB-4) score is a non-invasive method for evaluating liver fibrosis in patients with chronic liver disease, as it only requires the inclusion of age, ALT, AST, and PLT values on the physical examination form into the formula for calculation. For NAFLD, the critical values for liver fibrosis below grade 2 or above grade 3-4 are FIB-4<1.3 and FIB-4>2.67, respectively. The AST to platelet ratio index (APRI) is a diagnostic tool used to assess the degree of liver fibrosis in patients with chronic hepatitis B. It is calculated by dividing the AST level by the platelet count and multiplying the result by a correction factor. One important aspect in the interpretation of APRI scores is the determination of the upper limit of normal (ULN) value for AST. The ULN value represents the upper threshold of AST levels that are considered within the normal range. Immediately after blood collection, serum PIIINP levels were measured using the latex immunoturbidimetry method, and the reagent kits were supplied by Zhongshan Maisheng Medical Technology Co., Ltd., with strict adherence to the instructions.

Statistical Analysis

SPSS 22.0 (IBM Corp., Armonk, NY, USA) and R language software (Lucent Technologies Corp., Mount Jasmine, NJ, USA) were used for statistical analysis and graphical plotting of the data in this study. This study employed the Kolmogorov Smirnov (KS) test to evaluate data normality and utilized the Levene test to assess homogeneity of variance. Continuous data are expressed as mean ± standard deviation (SD), and the Welch Two Sample *t*-test was used to compare differences between groups. Counting data are expressed as ratios, and the Chi-square test was used to compare differences between groups. Diagnostic performance was analyzed using ROC curves to determine the Youden index, optimal predictive cut-off value, specificity, and sensitivity of serum PIIINP values. The statistical significance level was set at $\alpha=0.05$.

Results

Baseline Information

The patients' general demographic data is presented in Table I. No statistically significant differences were noted in the basic data between the two groups ($p>0.05$). When comparing liver enzyme markers between the two groups, the AST and ALT levels in the definite NASH group were significantly higher than those in the Not/borderline group ($p<0.05$). In terms of lipid parameters, the two groups showed similar serum concentrations of TC, LDL, HDL, and TG ($p>0.05$). Additionally, the HOMA-IR, FIB-4, and APRI indices, as well as the PIIINP level in the definite NASH group, were significantly higher than those in the Not/borderline group ($p<0.05$).

Relationship of Various NAFLD Characteristics with PIIINP

The relationship between different hepatocyte lesions, such as steatosis, lobular inflammation, hepatocyte ballooning, and PIIINP levels, is demonstrated in Figure 1. Plasma PIIINP levels markedly increased with the severity of steatosis,

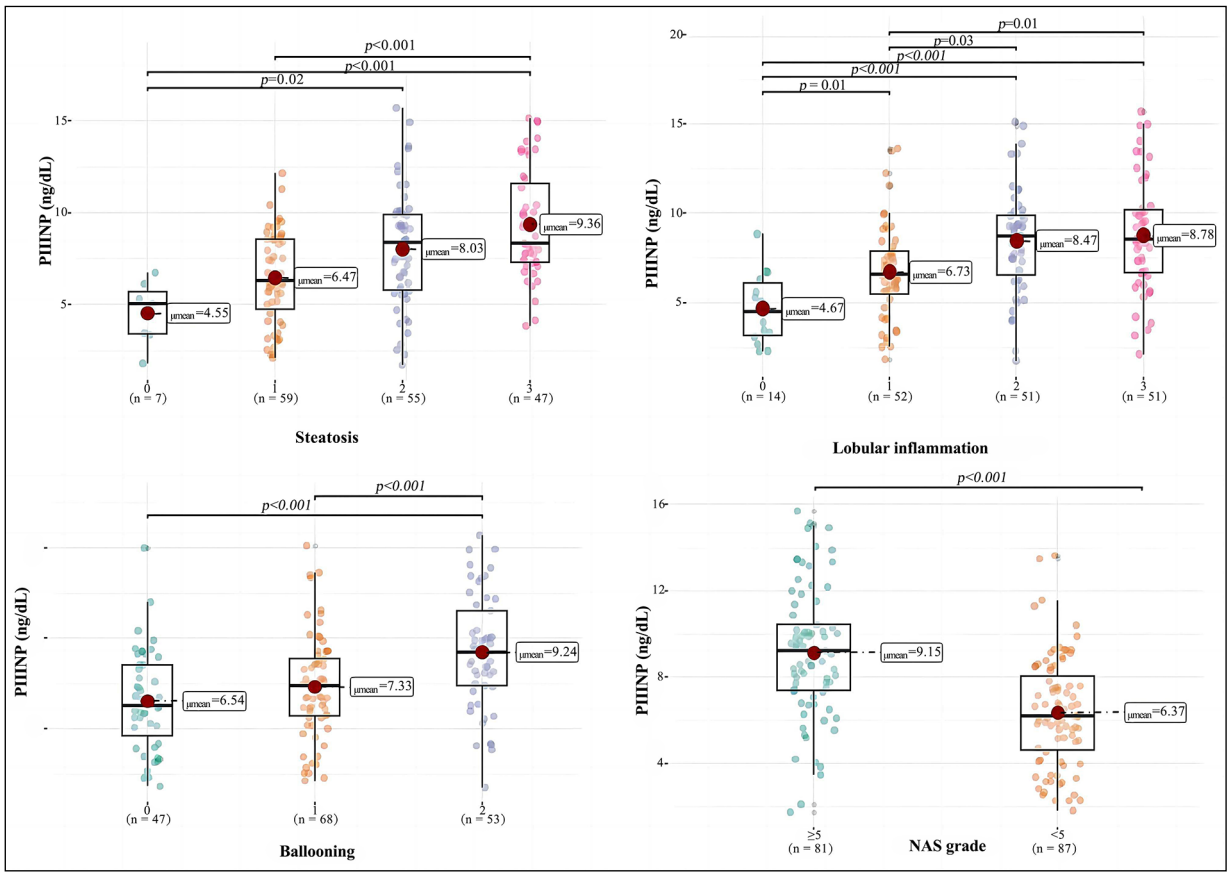

Figure 1. The relationship between PIIINP levels and steatosis, lobular inflammation, and hepatocellular ballooning.

Table I. Baseline information.

Characteristic	Not/borderline, N=87[1]	Definite, N=81[1]	*p*-value[2]
Age	56±25	59±23	0.485
Gender			0.782
Male	39 (45%)	39 (48%)	
Female	48 (55%)	42 (52%)	
BMI (Body Mass Index) (kg/m^2)	26±6	27±6	0.062
AST (aspartate transaminase) (IU/L)	27±7	30±7	0.008
ALT (alanine transaminase) (IU/L)	28.0±4.7	30.2±4.8	0.003
TC (total cholesterol) (mg/dL)	150±17	154±16	0.089
LDL (low density lipoprotein) (mg/dL)	86±15	90±17	0.145
HDL (high density lipoprotein) (mg/dL)	41±9	42±8	0.457
TG (triglycerides) (mg/dL)	89±24	94±16	0.115
HOMA-IR (homeostatic model assessment for insulin resistance) score	3.29±0.98	4.88±2.31	<0.001
FIB-4 (fibrosis biomarker-4) score	0.22±0.04	0.25±0.06	0.005
APRI (aminotransferase-to-platelet ratio index) score	0.19±0.04	0.21±0.05	0.001
PIIINP (propeptide type III collagen) (ng/dL)	6.4±2.5	9.1±3.0	<0.001

[1]Mean ± SD; n (%). [2]Welch Two Sample *t*-test; Chi-squared test.

lobular inflammation, and hepatocyte ballooning. Within steatosis, no significant difference was noted between the plasma PIIINP levels for scores 0 and 1 ($p>0.05$), but score 2 was significantly higher than score 1 ($p<0.05$), and score 3 was significantly higher than both scores 1 and 2 ($p<0.05$). As lobular inflammation scores increased, there was a notable rise in plasma PIIINP

Table II. Diagnostic performance for definite NASH.

	Sems	Spec	Youden's index	Cut-off point	AUC (95% CI)
PIIINP (propeptide type III collagen)	71.6%	73.6%	0.45	7.72	0.766 (95% CI: 0.694, 0.839)
APRI (aminotransferase-to-platelet ratio index)	60.5%	63.2%	0.24	0.21	0.634 (95% CI: 0.549, 0.718)
FIB-4 (fibrosis biomarker-4)	46.9%	79.3%	0.26	0.26	0.621 (95% CI: 0.0.534, 0.708)

levels ($p<0.05$), but no significant difference was seen between scores 3 and 2 ($p>0.05$). In ballooning, score 2 of plasma PIIINP levels were significantly higher than scores 0 and 1 ($p<0.05$), with no significant difference between scores 0 and 1 ($p>0.05$). In NAS, plasma PIIINP levels were significantly lower for NAS≤5 compared to NAS>5 ($p<0.05$).

Diagnostic Performance and Optimal Thresholds of Different Indices for NALFD Diagnosis

ROC curves were used to evaluate the diagnostic value of different indices for NAFLD, as shown in Figure 2. The AUC of PIIINP for diagnosing definite NASH was 0.766 (95% CI: 0.694, 0.839), with a cut-off value of 7.72. The AUC of APRI for diagnosing definite NASH was 0.634 (95% CI: 0.549, 0.718), with a cut-off value of 0.21. The AUC of FIB-4 for diagnosing definite NASH was 0.621 (95% CI: 0.534, 0.708), with a cut-off value of 0.26. The AUC of PIIINP for diagnosing definite NASH was significantly higher than that of APRI and FIB-4 ($p<0.05$), suggesting a better diagnostic efficiency.

When the respective cut-off values were used as diagnostic thresholds, PIIINP exceeding 7.72 ng/dL demonstrated a sensitivity of 71.6% and a specificity of 73.6% for diagnosing definite NASH, with a Youden's index of 0.45. For APRI greater than 0.21, the sensitivity and specificity for diagnosing definite NASH were 60.5% and 63.2%, respectively, with a Youden's index of 0.24; and for FIB-4 greater than 0.26, the sensitivity and specificity for diagnosing definite NASH were 46.9% and 79.3% respectively, with a Youden's index of 0.26. The outcomes related to diagnostic performance are presented in Table II.

Discussion

NAFLD emerges as the leading liver disease on a global scale, closely associated with IR and the cluster of conditions collectively known as metabolic syndrome. NASH, a subtype of NAFLD characterized by necrotic inflammatory changes, is primarily defined by NAS[12]. Patients with NAFLD commonly experience a subtle onset and gradual progression of liver disease.

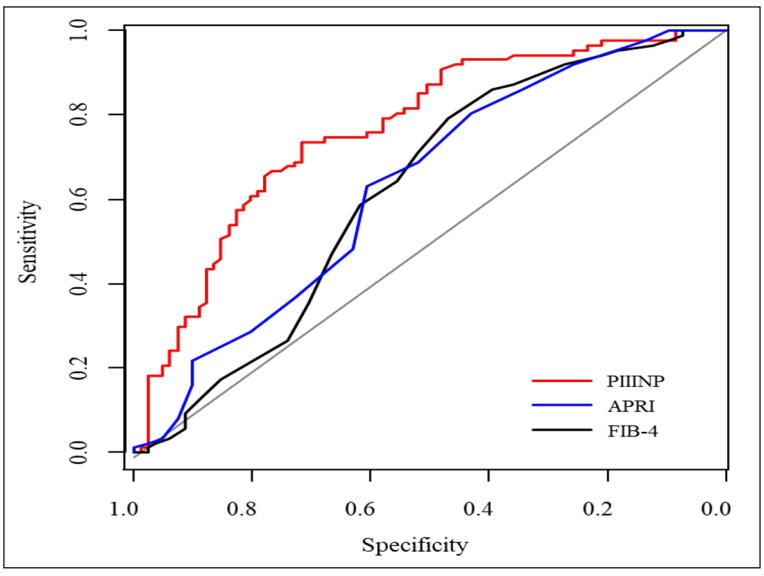

Figure 2. ROC curves for definite NASH.

Among those with NASH, the average progression is one stage of liver fibrosis every 7-10 years. It is important to note that bridging fibrosis and cirrhosis are independent predictors of adverse outcomes in NAFLD[13]. A longitudinal investigation encompassing 619 NAFLD patients demonstrated an independent correlation between the fibrosis staging of NAFLD and long-term survival, liver transplantation, and liver-related events, with no such association evident for other histological characteristics[14]. The severity of NAFLD presents a significant challenge for diagnosis and treatment. Histological analysis of liver biopsy is the gold standard for NASH diagnosis and staging and serves as a substantive contributor to predicting liver-related mortality. However, its invasiveness, expense, and the potential for variation in both sampling and interpretation restrict its widespread use[15,16]. Non-invasive, easy-to-use, and cost-effective alternatives to liver biopsy are required to better assess the severity of NAFLD[17,18]. Over the past decade, new circulating biomarkers have been evaluated[19] in NAFLD, and PIIINP has been a subject of significant discussion.

In the present study, the plasma PIIINP levels were 6.4±2.5 ng/mL for patients without NASH/borderline NASH and 9.1±3.0 ng/mL for those with definite NASH. Moreover, we demonstrated that a cut-off value >7.72 ng/mL for plasma PIIINP can differentiate definite NASH with a sensitivity of 71.6%, a specificity of 73.6%, and an ROC of 0.766 (95% CI: 0.694, 0.839). The increase in PIIINP may be subsequent to the increased collagen synthesis and/or degradation, and circulating levels of PIIINP can serve as a non-invasive biomarker for liver fibrosis. Plasma PIIINP levels have been shown[20] to be associated with various chronic liver diseases, including NAFLD. PIIINP is one of the most important ECM components in the liver. Through type III collagen synthesis, the N-terminal propeptide of type III procollagen is removed from type III procollagen, leading to the release of PIIINP into the bloodstream[21]. Hamza et al[22] reported that average serum PIIINP levels were higher in NAFLD patients than in controls, and PIIINP could serve as a marker of liver damage and fatty degeneration in obese children and adolescents, with a suggested cut-off value of 8.5 ng/mL, a sensitivity of 74%, and a specificity of 33%. Tanwar et al[23] demonstrated that in 172 biopsy-confirmed adult NAFLD patients, the ROC for plasma PIIINP levels to differentiate NASH from simple steatosis was 0.77-0.82, which was similar to the findings of our study.

The results of the current research showed a higher AUC of PIIINP for diagnosing NASH than that of APRI and FIB-4. APRI is a non-invasive diagnostic model that relies on just two commonly used laboratory indicators, rendering it straightforward to implement in clinical practice. While it demonstrates a positive correlation with liver fibrosis in NAFLD, its sensitivity is insufficient to fulfill clinical diagnostic requirements. As such, it primarily functions as a screening tool[24]. The FIB-4 index, originally devised as an assessment tool for the extent of hepatic fibrosis in patients concurrently affected by human immunodeficiency virus (HIV) and hepatitis C virus (HCV), has recently demonstrated a superior diagnostic efficacy relative to ultrasound methodologies for detecting liver fibrosis in patients with NAFLD. However, the intricate calculation formula of this model poses a challenge to its widespread adoption in primary healthcare settings[25]. In the 2017 guidelines[15], the American Association for the Study of Liver Diseases (AASLD) advocated the use of FIB-4 as an auxiliary tool in diagnosing patients with NAFLD who exhibit advanced fibrosis or cirrhosis. The findings of the current study showed that plasma PIIINP levels increased dramatically with the severity of fatty degeneration, lobular inflammation, and hepatocyte ballooning. Steatosis, inflammatory response, and hepatocyte injury (ballooning and/or pericellular fibrosis) are essential histological features for diagnosing NASH. Several studies[26,27] have confirmed the association of hepatocellular pathological changes with PIIINP, but the exact mechanism is still unclear.

There are certain limitations in this study as it has not undergone validation for biomarkers such as enhanced liver fibrosis (ELF), PRO-C3 (a neo-epitope pro-peptide of type III collagen formation), NIS4, and cytokeratin (CK)-18. The reason is that the enhanced liver fibrosis (ELF) test is a serological biomarker for the fibrosis stage of chronic liver disease, which accurately stages liver fibrosis independently of elevated transaminases as inflammatory markers. It has superior prognostic performance in the biopsy of alcohol-related liver disease, but currently, no clear definition and a gold standard for diagnosing fibrosis is available. PRO-C3 and NIS4 are commonly used biomarkers in NASH research. Elevated levels of PRO-C3 indicate

more prolonged liver fibrosis, though confirmation through liver biopsy is necessary. The NIS4 technology can be used to identify high-risk NASH patients with metabolic risk factors. However, its overall predictive accuracy and specificity of NIS4 are low for non-high-risk patients. In the case of high-risk NASH patients, its sensitivity is also comparatively low. CK-18 is mainly used to detect and predict drug-induced liver injury with higher sensitivity and specificity.

Conclusions

NASH patients in this study exhibited significantly elevated PIIINP serum levels, which were closely associated with hepatocyte pathological changes. PIIINP demonstrated superior competence in diagnosing NASH than APRI and FIB-4 and thus offers a viable alternative for the clinical diagnosis of NASH.

Conflict of Interest
The authors declare that they have no conflict of interest.

Informed Consent
Written informed consents were obtained from patients.

Ethics Approval
The study was approved by the First Hospital of Hebei Medical University Ethical approval No.: FHHMU-105214).

Funding
Not received.

Acknowledgments
We thank the First Hospital of Hebei Medical University for their help in the information collection for the present project.

Authors' Contributions
Y.M. edited the manuscript and guaranteed the integrity of the entire study. X.Y. and R.L. collected data. J.Z. and H.Y. processed the data and the statistics. Z.Z. and S.J. reviewed the manuscript and revised the results and discussion sections, respectively. Y.W. designed the research, provided critical comments, and revised the manuscript. All authors contributed to the article and approved the submitted version.

Data Availability
The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

ORCID ID
Yujing Mi: 0009-0009-9209-1037
Zheng Zhang: 0009-0008-0567-8212
Suli Jin: 0000-0002-4878-150X
Huimin Yan: 0009-0003-9882-4584
Xuemei Yang: 0009-0000-0988-5990
Jing Zhao: 0000-0001-5042-9807
Rongxin Liu: 0009-0005-2168-8742
Yan Wu: 0009-0002-8642-8261

References

1) Kim CH, Younossi ZM. Nonalcoholic fatty liver disease: a manifestation of the metabolic syndrome. Cleve Clin J Med 2008; 75: 721-728.
2) Abd El-Kader SM, El-Den Ashmawy EM. Non-alcoholic fatty liver disease: The diagnosis and management. World J Hepatol 2015; 7: 846-858.
3) Rinella ME. Nonalcoholic fatty liver disease: a systematic review. JAMA 2015; 313: 2263-2273.
4) 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: 73-84.
5) Diehl AM, Day C. Cause, Pathogenesis, and Treatment of Nonalcoholic Steatohepatitis. N Engl J Med 2017; 377: 2063-2072.
6) Duprez DA, Gross MD, Sanchez OA, Kizer JR, Ix JH, Lima J, Tracy RP, Jacobs DR, Jr. Collagen Turnover Markers in Relation to Future Cardiovascular and Noncardiovascular Disease: The Multi-Ethnic Study of Atherosclerosis. Clin Chem 2017; 63: 1237-1247.
7) Pettersson-Pablo P, Samyn D, Wasim J, Vink M. Reference interval for type III procollagen (PIIINP) using the Advia centaur PIIINP assay in adults and elderly. Scand J Clin Lab Invest 2021; 81: 649-652.
8) Lieber CS, Weiss DG, Paronetto F. Value of fibrosis markers for staging liver fibrosis in patients with precirrhotic alcoholic liver disease. Alcohol Clin Exp Res 2008; 32: 1031-1039.
9) Dong H, Xu C, Zhou W, Liao Y, Cao J, Li Z, Hu B. The combination of 5 serum markers compared to FibroScan to predict significant liver fibrosis in patients with chronic hepatitis B virus. Clin Chim Acta 2018; 483: 145-150.
10) Guzzo-Merello G, Segovia J, Dominguez F, Cobo-Marcos M, Gomez-Bueno M, Avellana P, Millan I, Alonso-Pulpon L, Garcia-Pavia P. Natural

history and prognostic factors in alcoholic cardiomyopathy. JACC Heart Fail 2015; 3: 78-86.

11) Jahromi MK, Daftari G, Farhadnejad H, Tehrani AN, Teymoori F, Salehi-Sahlabadi A, Mirmiran P. The association of healthy lifestyle score and risk of non-alcoholic fatty liver disease. BMC Public Health 2023; 23: 973.
12) Ekstedt M, Hagström H, Nasr P, Fredrikson M, Stål P, Kechagias S, Hultcrantz R. Fibrosis stage is the strongest predictor for disease-specific mortality in NAFLD after up to 33 years of follow-up. Hepatology 2015; 61: 1547-1554.
13) Heinle JW, DiJoseph K, Sabag A, Oh S, Kimball SR, Keating S, Stine JG. Exercise Is Medicine for Nonalcoholic Fatty Liver Disease: Exploration of Putative Mechanisms. Nutrients 2023; 15: 2452.
14) Angulo P, Kleiner DE, Dam-Larsen S, Adams LA, Bjornsson ES, Charatcharoenwitthaya P, Mills PR, Keach JC, Lafferty HD, Stahler A, Haflidadottir S, Bendtsen F. Liver Fibrosis, but No Other Histologic Features, Is Associated With Long-term Outcomes of Patients With Nonalcoholic Fatty Liver Disease. Gastroenterology 2015; 149: 389-397.e310.
15) Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, Harrison SA, Brunt EM, Sanyal AJ. The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. Hepatology 2018; 67: 328-357.
16) Jennison E, Byrne CD. Recent advances in NAFLD: current areas of contention. Fac Rev 2023; 12: 10.
17) Xu Q, Feng M, Ren Y, Liu X, Gao H, Li Z, Su X, Wang Q, Wang Y. From NAFLD to HCC: Advances in noninvasive diagnosis. Biomed Pharmacother 2023; 165: 115028.
18) Jang W, Song JS. Non-Invasive Imaging Methods to Evaluate Non-Alcoholic Fatty Liver Disease with Fat Quantification: A Review. Diagnostics (Basel) 2023; 13: 1852.
19) Watt J, Kurth MJ, Reid CN, Lamont JV, Fitzgerald P, Ruddock MW. Non-alcoholic fatty liver disease-A pilot study investigating early inflammatory and fibrotic biomarkers of NAFLD with alcoholic liver disease. Front Physiol 2022; 13: 963513.
20) Murawaki Y, Nishimura Y, Ikuta Y, Idobe Y, Kitamura Y, Kawasaki H. Plasma transforming growth factor-beta 1 concentrations in patients with chronic viral hepatitis. J Gastroenterol Hepatol 1998; 13: 680-684.
21) Kuivaniemi H, Tromp G. Type III collagen (COL3A1): Gene and protein structure, tissue distribution, and associated diseases. Gene 2019; 707: 151-171.
22) Hamza RT, Ahmed AY, Rezk DG, Hamed AI. Dietary fructose intake in obese children and adolescents: relation to procollagen type III N-terminal peptide (P3NP) and non-alcoholic fatty liver disease. J Pediatr Endocrinol Metab 2016; 29: 1345-1352.
23) Tanwar S, Trembling PM, Guha IN, Parkes J, Kaye P, Burt AD, Ryder SD, Aithal GP, Day CP, Rosenberg WM. Validation of terminal peptide of procollagen III for the detection and assessment of nonalcoholic steatohepatitis in patients with nonalcoholic fatty liver disease. Hepatology 2013; 57: 103-111.
24) Pennisi G, Enea M, Falco V, Aithal GP, Palaniyappan N, Yilmaz Y, Boursier J, Cassinotto C, de Lédinghen V, Chan WK, Mahadeva S, Eddowes P, Newsome P, Karlas T, Wiegand J, Wong VW, Schattenberg JM, Labenz C, Kim W, Lee MS, Lupsor-Platon M, Cobbold JFL, Fan JG, Shen F, Staufer K, Trauner M, Stauber R, Nakajima A, Yoneda M, Bugianesi E, Younes R, Gaia S, Zheng MH, Cammà C, Anstee QM, Mózes FE, Pavlides M, Petta S. Noninvasive assessment of liver disease severity in patients with nonalcoholic fatty liver disease (NAFLD) and type 2 diabetes. Hepatology 2023; 78: 195-211.
25) Meng Q, Zhang M. Clinical Significance of Serum Collagen Type IV and Procollagen Type III N-Peptide Levels in Diagnosis and Differential Diagnosis of Lymphedema. Lymphat Res Biol 2023; 21: 8-14.
26) Kirik A, Dogru T, Yanik B, Sen H, Eroglu M, Baykan O, Bozyel EA, Ergene A, Selçuk E, Tasci I, Sonmez A. The relationship of circulating MOTS-c level with liver fibrosis and metabolic components in patients with metabolic dysfunction-associated fatty liver disease. Eur Rev Med Pharmacol Sci 2023; 27: 8074-8080.
27) Alarfaj SJ, Bahaa MM, Yassin HA, El-Khateeb E, Kotkata FA, El-Gammal MA, Elberri AI, Habba E, Zien El-Deen EE, Khrieba MO, El-Masry TA, Negm WA, Elberri EI. A randomized placebo-controlled, double-blind study to investigate the effect of a high oral loading dose of cholecalciferol in non-alcoholic fatty liver disease patients, new insights on serum STAT-3 and hepassocin. Eur Rev Med Pharmacol Sci 2023; 27: 7607-7619.