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Research Letter

The Reduced Accuracy of Non-invasive Tests for Significant Fibrosis in Chronic Hepatitis B Patients with Metabolic Dysfunction-associated Steatotic Liver Disease

Fajuan Rui[1,2#], Wenjing Ni[1,2#], Yee Hui Yeo[3], Youwen Tan[4], Liang Xu[5,6,7], Junping Shi[8*] and Jie Li[1,2*]

[1]Department of Infectious Diseases, Nanjing Drum Tower Hospital Clinical College of Nanjing University of Chinese Medicine, Nanjing, Jiangsu, China; [2]Department of Infectious Disease, Nanjing Drum Tower Hospital, Affiliated Hospital of Medical School, Nanjing University, Nanjing, Jiangsu, China; [3]Karsh Division of Gastroenterology and Hepatology, Cedars-Sinai Medical Center, Los Angeles, CA, USA; [4]The Third Hospital of Zhenjiang Affiliated Jiangsu University, School of Medicine, Jiangsu University, Zhenjiang, Jiangsu, China; [5]Clinical School of the Second People's Hospital, Tianjin Medical University, Tianjin, China; [6]Department of Hepatology, Tianjin Second People's Hospital, Tianjin, China; [7]Tianjin Research Institute of Liver Diseases, Tianjin, China; [8]Department of Infectious Diseases, The Affiliated Hospital of Hangzhou Normal University, Hangzhou, Zhejiang, China

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Metabolic dysfunction-associated steatotic liver disease (MASLD), formerly known as non-alcoholic fatty liver disease (NAFLD), is increasingly prevalent in Asia,[1] an area also endemic for chronic hepatitis B (CHB). Our previous study showed that the coexistence of CHB and hepatic steatosis (HS) has reached 36.5% in Asia,[2] with a similar figure observed globally.[3] This coexistence may exacerbate the progression of hepatic fibrosis. Non-invasive tests (NITs) like the fibrosis-4 index (FIB-4),[4] aspartate aminotransferase-to-platelet ratio index (APRI),[5] and the NAFLD fibrosis score (NFS)[6] are crucial for diagnosing hepatic fibrosis. While previous studies have revealed that these NITs have suboptimal accuracy for diagnosing hepatic fibrosis,[7–9] few have explored the impact of different numbers of cardiometabolic risk factors (CMRFs) on diagnostic accuracy among patients with CHB and MASLD. Therefore, we aimed to evaluate the accuracy of these NITs in identifying significant hepatic fibrosis in this specific population.

This multi-center, retrospective study was conducted at eleven Chinese hospitals (ClinicalTrials.gov identifier: NCT05766449). CHB was defined as hepatitis B surface antigen positive for ≥6 months. HS was diagnosed as steatosis exceeding 5% confirmed by liver biopsy. In this study, MASLD was defined as biopsy-proven HS in combination with one or more of the following CMRFs: (1) BMI ≥ 25 kg/m^2; (2) fasting plasma glucose ≥5.6 mmol/L or diagnosed type 2 diabetes mellitus (T2DM); (3) hypertension; (4) plasma triglyceride ≥1.70 mmol/L; (5) plasma high-density lipoprotein cholesterol ≤1.0/1.3 mmol/L for males/females. The exclusion criteria included: (1) excessive alcohol intake (ongoing or recent alcohol consumption over 30/20 g of alcohol per day for men/women)[10]; (2) co-infection with other viruses; (3) presence of other liver diseases; (4) diagnosis of malignancies; (5) insufficient clinical data. Liver fibrosis was categorized into S0-S4 according to Scheuer's classification, with ≥S2 defined as significant fibrosis.[11] To ensure data relevancy and consistency, only laboratory examinations conducted within a 14-day window before the liver biopsy were considered. The population was classified into three groups: group A of CHB patients with simple HS, group B of CHB patients with MASLD involving 1–3 CMRFs, and group C of CHB patients with MASLD involving 4–5 CMRFs. The different optimal cut-off values of FIB-4, NFS, and APRI in the three groups were determined by the Youden index. Differences in the area under the curve (AUC) were compared using the DeLong test.

A total of 1,063 eligible patients were enrolled, with 47 patients (53.4%), 406 patients (46.3%), and 50 patients (51.0%) in groups A, B, and C diagnosed as ≥S2 confirmed by liver biopsy, respectively. The population characteristics of the whole cohort are available in Supplementary Table 1. The optimal cutoff values of the three NITs, along with the corresponding sensitivity, specificity, positive predictive value, and negative predictive value in the three groups, are presented in Supplementary Table 2. For diagnostic performance, the AUCs of FIB-4, APRI, and NFS in diagnosing ≥S2 within group A were 0.78, 0.78, and 0.72, respectively (Fig. 1). However, in CHB patients with MASLD, the accuracy of the three NITs decreased with an increasing number of CMRFs. The AUCs of FIB-4, APRI, and NFS were 0.65, 0.68, and

[#]Contributed equally to this work.
[*]**Correspondence to:** Jie Li, Department of Infectious Disease, Nanjing Drum Tower Hospital, Affiliated Hospital of Medical School, Nanjing University, Nanjing, Jiangsu 210000, China. ORCID: https://orcid.org/0000-0003-0973-8645. Tel: +86-25-83106666, Fax: +86-25-83626060, E-mail: lijier@nju.edu.cn; Junping Shi, Department of Infectious Diseases, The Affiliated Hospital of Hangzhou Normal University, Hangzhou, Zhejiang 310015, China. ORCID: https://orcid.org/0000-0001-9434-897X. Tel: +86-571-88358060, Fax: +86-571-88021730, E-mail: 20131004@hznu.edu.cn

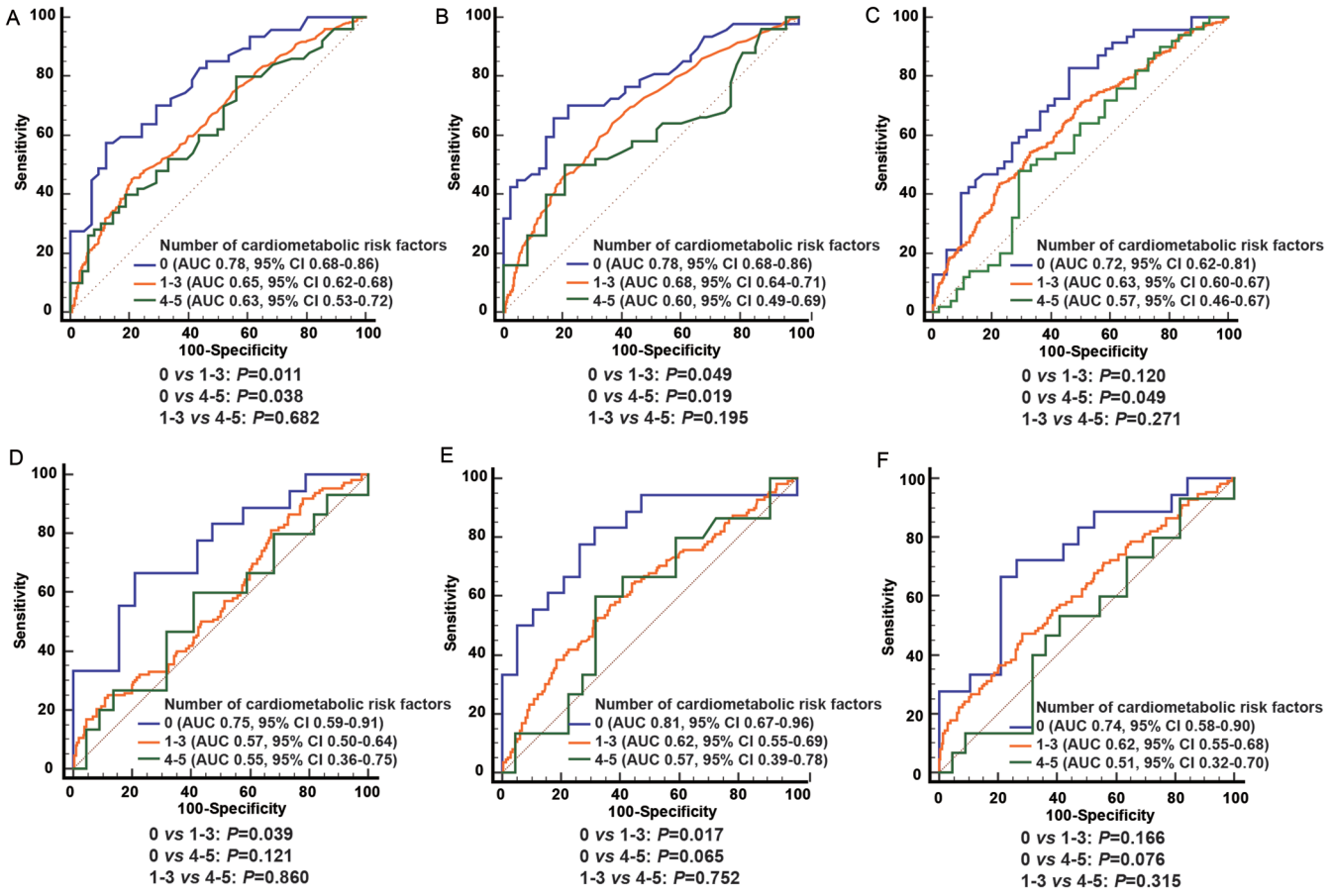

Fig. 1. The diagnostic performance of the three non-invasive tests for significant fibrosis (≥S2) in CHB patients with HS or MASLD. (A) The AUC of FIB-4 for all patients, (B) The AUC of APRI for all patients, (C) The AUC of NFS for all patients, (D) The AUC of FIB-4 in patients with normal ALT levels, (E) The AUC of APRI in patients with normal ALT levels, (F) The AUC of NFS in patients with normal ALT levels. Normal ALT levels are defined as ALT < 40 IU/L. HS, hepatic steatosis; MASLD, metabolic dysfunction-associated steatotic liver disease; FIB-4, the fibrosis-4 index; APRI, the aspartate aminotransferase-to-platelet ratio index; NFS, the non-alcoholic fatty liver disease fibrosis score; AUC, area under the curve; CHB, chronic hepatitis B; ALT, alanine aminotransferase.

0.63 in group B, respectively. These AUCs further declined in group C, all falling below 0.63. FIB-4 and APRI both demonstrated significantly poorer diagnostic performance for group B and group C compared to group A (all $p < 0.05$, Fig. 1), and the AUC of NFS was significantly lower in group C compared with group A ($p < 0.05$, Fig. 1). In the subgroup with normal ALT levels (<40 IU/L), a consistent trend was observed. As the number of CMRFs increased, the AUC of the three NITs decreased. Significant differences were achieved when comparing the AUCs of FIB-4 and APRI between groups A and B (both $p < 0.05$, Fig. 1).

In this study, we found that the diagnostic accuracy of the three NITs diminished as the number of CMRFs increased in CHB patients with MASLD. NITs have emerged as valuable tools for diagnosing and prognosing chronic liver disease. However, the diagnostic accuracy can vary when patients have different CMRFs. A retrospective study by Boursier J *et al*.[12] reported that the AUCs of FIB-4 and NFS for identifying advanced fibrosis were significantly lower in NAFLD patients with T2DM compared to those without T2DM. Furthermore, an individual patient data meta-analysis involving 5,735 NAFLD patients confirmed that FIB-4 and NFS performed better in patients with lower BMI and without T2DM.[13] These findings align with our results, suggesting that CMRFs potentially impact the diagnostic performance of NITs. The three existing NITs mainly utilize transaminase levels, platelet counts, and other available variables that indirectly reflect liver injury. Since MASLD often lacks specific symptoms for a long period, scores calculated from these NITs with limited specificity may mask the progression of liver disease. In the context of CHB combined with MASLD, metabolic dysregulation could exacerbate liver fibrosis. Hepatocyte damage may result in the release of inflammatory cytokines, triggering hepatic stellate cell activation and proliferation.[14] Excessive fat accumulation and oxidative stress can promote pro-fibrotic and carcinogenic processes.[14] Abnormal lipid metabolism induced by HBsAg-related promyelocytic leukemia protein deficiency may accelerate the progression of liver-related adverse events.[14] The complex interplay among CHB, MASLD, and other CMRFs necessitates further investigation.

Currently, some biomarkers or panels have been developed to directly reflect the severity of liver fibrosis by measuring circulating markers released during fibrogenesis or extracellular matrix remodeling, such as N-terminal propeptide of type 3 collagen, enhanced liver fibrosis, and FibroTest.[15] These novel tests show promise for more precise diagnosis in populations with chronic liver disease.

This study has some limitations. Firstly, as a retrospective study, the data were collected before the nomenclature of MASLD was proposed. Therefore, some indicators used to

evaluate CMRFs are missing, such as waist circumference and two-hour postprandial blood glucose. Secondly, the sample sizes of group A (0 CMRF) and group C (4–5 CMRFs) are too small, limiting the potential for more detailed analysis. Thirdly, pathological signs of steatohepatitis, such as hepatic ballooning or Mallory bodies, were not strictly recorded in patients.

In conclusion, this study showed a notable decrease in the diagnostic accuracy of three NITs for significant fibrosis in patients with concomitant MASLD and CHB, especially in those with an elevated number of CMRFs. Future research is needed to improve the diagnostic performance of NITs in this population. Developing personalized algorithms for specific patients and exploring the impact of various metabolic factors on NITs are necessary to optimize liver fibrosis risk stratification in CHB patients with MASLD.

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Conflict of interest

JL has been an Editorial Board Member of *Journal of Clinical and Translational Hepatology* since 2024. The other authors have no conflict of interests related to this publication.

Author contributions

Study design and conceptualization (JL, JS, YHY), data analysis and manuscript drafting, (FR, WN), data extraction and interpretation (YT, LX), and manuscript revision and editing (JL, JS). All authors have read and approved the final manuscript.

Ethical statement

This multi-center, retrospective study was conducted at eleven Chinese hospitals (ClinicalTrials.gov identifier: NCT05766449). We have received approval from the Institutional Ethics Review Board of all the involved hospitals, with document numbers of 2008022. This study was carried out in accordance with the Declaration of Helsinki. The informed consent was waived for a retrospective study.

Data sharing statement

We are unable to provide access to our data for privacy reasons. The protocol and statistical analysis methods used in the study can be requested directly from the corresponding author after approval.

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