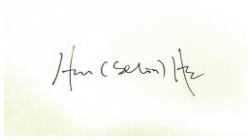
# Certificate from *Medjaden* Bioscience Limited

Dear Sir or Madam:

This document certifies that the manuscript, **A novel noninvasive model based on serum ceruloplasmin to predict liver fibrosis in hepatitis B virus-infected patients with persistently normal serum ALT**, was edited for proper English language, grammar, punctuation, spelling, and overall style by highly qualified native English speaking and professional scientific editors at *Medjaden* Bioscience Limited on 17th Mar. 2020. Neither the research content nor the authors' intentions were altered in any way during the editing process.

Yours sincerely,



(Ms.) Hua (Selin) He

for *Medjaden*

*Medjaden* Bioscience Limited
General Office
Address: 11415 Ashford Willow, Sugar Land, Texas, 77478, USA
Email: medjaden@gmail.com
Website: www.medjaden.com

**Information about *Medjaden Bioscience Limited*:**
*Medjaden* is an emerging editing company that provides professional services for laboratory researchers, postgraduates, and clinical doctors. Our mission is to help our clients initiate, maximize and accelerate the production of articles from their research work. The company employs experienced medical statisticians, writers, and editors drawn from both Chinese and English backgrounds worldwide, who strive to ensure that every edited article is published in an English journal, preferably one that is included in the Web of Science (WOS), the Science Citation Index (SCI) Expanded, Medline, PubMed, EMBASE, and other scientific resources.

**A novel noninvasive model based on serum ceruloplasmin to predict liver fibrosis in hepatitis B virus-infected patients with persistently normal serum ALT**

Na-Ling Kang, Jie-Min Zhang, Meng-Xin Lin, Xu-Dong Chen, Zu-Xiong Huang, Yue-Yong Zhu, Yu-Rui Liu, Da-Wu Zeng

**Na-Ling Kang, Yu-Rui Liu, Yue-Yong Zhu, Da-Wu Zeng,** Liver Center, The First Affiliated Hospital, Fujian Medical University, China, 350005

**Jie-Min Zhang,** Department of Pharmacy, The First Affiliated Hospital, Fujian Medical University, No. 20 Chazhong Road, Taijiang District, Fuzhou, Fujian, China, 350005

**Meng-Xin Lin**, Department of Infectious Diseases, The First Hospital of Quanzhou Affiliated with Fujian Medical University, No. 250 Dong Road, Licheng District, Quanzhou, Fujian, China, 362000

**Xu-Dong Chen**, Department of Gastroenterology, the 910th Hospital of the People's Liberation Army, No. 180 HuaYuan Road, Fengzhe District, Quanzhou, Fujian, China, 362000

**Zu-Xiong Huang**, Hepatology, Mengchao Hepatobiliary Hospital of Fujian Medical University, No. 312 Xihong Road, Gulou District, Fuzhou, Fujian, China, 350005

**Author Contributions:** Kang NL, Zeng DW and Liu YR conceived and designed the experiments; Kang NL, Zhang JM and Zeng DW performed the experiments; Chen XD, Huang ZX, Lin MX, Zhang JM and Zhu YY analyzed the data; Kang NL, Zhang JM, Chen XD, Huang ZX and Lin MX Contributed reagents/materials/analysis tools; Kang NL, Zhang JM and Zeng DW wrote the manuscript.

**Supported by** the Science and Technology Department of Fujian Province, No. 2018J01164; Quanzhou Science and Technology Bureau Planning Project, No. 2019N019S.

**Correspondence to: Dr. Da-Wu Zeng, MD,** Liver Center, The First Affiliated Hospital, Fujian Medical University, No. 20 Chazhong Road, Taijiang District, Fuzhou, Fujian, China, 350005; Zengdw1980@fjmu.edu.cn.

Tel: +86 156-05917968

**Abstract**

***BACKGROUND***

The presence of significant liver fibrosis in hepatitis B virus (HBV)-infected individuals with persistently normal serum ALT levels (PNALT) is a strong indicator for initiating antiviral therapy. Serum ceruloplasmin (CP) is negatively correlated with liver fibrosis in HBV-infected individuals.

***AIM***

To examine the potential value of CP and develop a noninvasive index including CP to assess significant fibrosis among HBV-infected individuals with PNALT.

***METHODS***

Two hundred and seventy-five HBV-infected individuals with PNALT were retrospectively evaluated. The association between CP and fibrotic stages was statistically analyzed. A predictive index including CP was constructed to predict significant fibrosis and compared to previously reported models.

***RESULTS***

Serum CP had an inverse correlation with liver fibrosis (r=-0.600). Using CP, the areas under the curves (AUCs) to predict significant fibrosis, advanced fibrosis, and cirrhosis were 0.774, 0.812, and 0.853, respectively. The ceruloplasmin hepatitis B virus (CPHBV)

model was developed using CP, platelet, and HBsAg levels to predict significant fibrosis. The AUCs of significant fibrosis, advanced fibrosis, and cirrhosis were 0.842, 0.920, and 0.904. CPHBV was superior to previous models like the Fibrosis-4 score, Forn's score, and S-index in predicting significant fibrosis in HBV-infected individuals with PNALT.

***CONCLUSION***

CPHBV could accurately predict liver fibrosis in HBV-infected individuals with PNALT. Therefore, CPHBV can be a valuable tool for antiviral treatment decisions.

**Keywords:** ceruloplasmin; liver fibrosis; chronic hepatitis B infection.

**Core tip:** Chronic HBV-infected individuals with PNALT may develop severe liver fibrosis, which requires antiviral therapy. Following up on our previous findings, this multicenter, cross-sectional study showed that CP has an inverse correlation with liver fibrosis and is a promising predictive marker for liver fibrosis among HBV-infected individuals with PNALT. We developed a noninvasive model using CP, platelet, and HBsAg levels to identify the various stages of fibrosis among HBV-infected individuals with PNALT. Our model could reduce the need for liver biopsy before antiviral treatment.

## INTRODUCTION

Approximately 292 million people have experienced hepatitis B virus (HBV) infection worldwide; nonetheless, less than 5% of those infected received antiviral therapy [1]. Chronic HBV infection can cause fibrosis, which can develop into hepatocellular carcinoma [2]. Therefore, timely diagnosis of liver fibrosis is instrumental for commencing anti-HBV treatment, which will ultimately control the progression of liver injury and improve the patient prognosis [3]. Assessment of serum alanine aminotransferase (ALT) level is a relatively inexpensive biochemical test that is widely used to detect liver injury. However, recent evidence suggests that some patients with normal ALT can also suffer from severe liver fibrosis [4-6].

Published guidelines for HBV management recommend that antiviral therapy be offered to chronic HBV-infected individuals with persistently normal ALT (PNALT) upon significant histological alterations [7-9]. Liver biopsy (LB) is a standard procedure in diagnosing hepatic fibrosis; however, its invasiveness increases the risk of complications [10-12]. FibroScan is another feasible alternative to LB due to its excellent diagnostic value in liver fibrosis [13-15]. However, its high cost and limitations in immune-tolerant HBV-infected individuals hinder its wide clinical application. The aspartate aminotransferase (AST)-to-platelet (PLT) ratio index (APRI) and fibrosis-4 score (FIB-4) have been recommended as noninvasive predictive indexes to assess liver fibrosis [16]. However, recent research showed that APRI and FIB-4 had poor diagnostic value for assessing the improvement of liver fibrosis during anti-HBV therapy [17]. Further, APRI and FIB-4 could not precisely estimate the stages of fibrosis in HBV-infected individuals with PNALT [18]. Therefore, it is essential to develop a novel predictive index to diagnose hepatic fibrosis in HBV-infected individuals with PNALT.

Serum ceruloplasmin (CP), a glycoprotein secreted by hepatocytes, carries more than 95% of the circulating copper in a healthy human. Studies have shown that CP has a strong antioxidant function and suppresses lipid peroxidation by eliminating superoxide anions [19, 20]. Further, abnormal CP levels have been implicated in other pathological conditions [21]. Our previous data showed that CP was negatively correlated with liver fibrosis, suggesting that CP is a useful marker to diagnose liver fibrosis in CHB individuals [22-24]. Nonetheless, the association between CP and hepatic fibrosis among HBV-infected individuals with PNALT remains poorly understood [23]. Therefore, our purpose was to develop a novel panel to noninvasively predict hepatic fibrosis among

HBV-infected individuals with PNALT using the CP levels. For this purpose, we routinely collected clinical data in a multicenter and cross-sectional study to develop the CPHBV model. Next, we compared the diagnostic value of the new panel with previously established parameters like APRI, FIB-4, the gamma-glutamyl transpeptidase-to-platelet ratio (GPR), S-index, and Forn's index [25-29].

## MATERIALS AND METHODS

### *Study population*

Two hundred and seventy-five HBV-infected individuals with PNALT were retrospectively assessed between June, 2010 and November, 2019 from three affiliated hospitals of Fujian Medical University [First Affiliated Hospital, Meng Chao Hepatobiliary Hospital (Xihong Branch of the First Affiliated Hospital) and The First Hospital of Quanzhou]. A portion of our patient cohort was previously investigated in former studies. In particular, 15.1% of the patients were investigated in Zeng DW et al., 2013 [22] and 31.1% of the patients were investigated in Zeng DW et al., 2016 [23]. All treatment-naive patients had been HBsAg-positive for more than 6 months. All exclusion criteria presented in our previous paper were applied in this study [30]. All individuals were randomly stratified into a training and a validation group. This study was approved by the Institutional Review Board of Fujian Medical University, and the need for informed consent was waived due to the retrospective nature of the study.

### *Quantification of liver fibrosis*

Liver specimens were obtained using aspiration 16-gauge modified needles (TSK Laboratory, Tochigi, Japan). Qualified liver specimens (a length of more than 1.5 cm and

6 portal tracts) were obtained, fixed in 4% formalin, embedded in paraffin, and processed with hematoxylin-eosin-safran, and Masson's trichrome according to the standard protocols. Liver fibrosis staging (F0-F4) was carried out according to the METAVIR scoring system by a pathologist blinded to the patients' data. Significant fibrosis was defined as F≥2, advanced fibrosis as F≥3, and cirrhosis as F=4, as detailed previously [31].

***Serum CP and other clinical parameters***

The serum CP was examined by use of the nephelometric immunoassay kit (BN II System, Siemens Healthcare Diagnostics GmbH, Eschborn, Germany). Quantitative HBsAg was tested by use of the Elecsys® HBsAg II quant assay (Roche Diagnostics, Mannheim, Germany) or the Abbott ARCHITECT® assay (Abbott Laboratories, Chicago), and HBV-DNA was examined by use of quantitative polymerase chain reaction assay (PG Co, Shenzhen, China). Other routine biochemical parameters were assayed using an automatic biochemistry analyzer. Laboratory tests were assessed 1 week prior to the LB procedure.

***Statistical analysis***

The Student's t-test was utilized to investigate differences in continuous normal distribution variables. We performed the Mann–Whitney test to investigate the differences in continuous non-normal distribution variables. The Chi-Square test was used to detect differences in categorical data. The Spearman test for correlation analyses was applied. Univariate and multivariate regression analyses were applied to select independent parameters linked with significant liver fibrosis. Receiver operating characteristic (ROC) curve analysis was carried out to obtain the best cut-off value of CP

for liver fibrosis. Diagnostic accuracy was obtained with the area under the curve (AUC). To compare the AUC of CPHBV with that of five noninvasive models (APRI, FIB-4, GPR, Forn's index, and S index), the Z test was applied. Statistical analysis was applied by use of SPSS v23.0.

## RESULTS

### *Demographic and clinical characteristics*

Among a total of 275 enrolled patients (mean age=40.25 ± 9.65 years), 194 (70.5%) were men and 71 (29.5%) were women (Table 1). Further, 54.5% of the patients presented with at least moderate liver necroinflammation (G≥2) or fibrosis (F≥2) and 19.3% had liver cirrhosis (F4). Analysis of the demographic and clinical features did not reveal significant differences between the training and validation groups ($P$>0.05, Table 1).

### Diagnostic value of CP for detecting different stages of liver fibrosis among HBV-infected individuals with PNALT

Serum CP levels revealed an inverse correlation with hepatic fibrosis ($r$=-0.6). The AUCs were 0.774 for F≥2, 0.812 for F≥3, and 0.853 for F4 (Table 2). Further, the best diagnostic CP values were 203.5 mg/L for F≥2, 190.5 mg/L for F≥3, and 182.5 mg/L for F4 (Table 2).

### Development of a novel panel for liver fibrosis

Next, we analyzed the correlation between various biochemical parameters and significant fibrosis (Table 3). Univariate analysis revealed that the CP, albumin, gamma glutamyl transpeptidase (GGT), total cholesterol (TCHO), cholinesterase (CHE), HBsAg, and PLT levels were different between individuals with non-significant and significant

fibrosis ($P$<0.05). These variables were then subjected to multivariate regression. CP, PLT, and HBsAg were identified as independent predictors. Using these parameters, a novel diagnostic model named CPHBV was developed to evaluate significant fibrosis in HBV-infected individuals with PNALT as follows: 37.122-10.072 × Log CP (mg/L) -4.291 × Log PLT ($10^9$/L) -0.958 × Log HBsAg (IU/mL).

**Accuracy of the new indexes in assessment of F≥2, F≥3, and F4**

We then analyzed the diagnostic value of the CPHBV model for detecting F≥2, F≥3, and F4 (Table 4). The AUC of CPHBV for predicting F≥2 was 0.875. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were 81.1%, 71.9%, 76.9%, and 76.7%, respectively, in the training group, and 70.3%, 80.3%, 82.1%, and 71.0% in the validation group. The AUC of CPHBV for the evaluation of significant fibrosis showed a comparable predictive value between the training and validation groups ($Z$=0.746, $P$=0.46). Upon using our model to assess advanced fibrosis and cirrhosis, the AUC, sensitivity, specificity, PPV, and NPV were 0.92, 90.9%, 79.6%, 67.8%, and 94.9%, respectively, for F≥3, and 0.904, 96.2%, 71.4%, 43.9%, and 98.9% for F4 in the training group (Table 4).

We further analyzed the predictive value of the CPHBV model for detecting F≥2, F≥3, and F4 in HBeAg-positive patients and HBeAg-negative patients (Table 5). The AUC of CPHBV for predicting F≥2 was 0.829. The sensitivity, specificity, PPV, and NPV were 74.2%, 83.2%, 83.6%, and 74.6% in HBeAg-positive patients, and 57.5%, 89.9%, 87.7%, and 62.6% in HBeAg-negative patients, respectively. Upon using our model to predict advanced fibrosis and cirrhosis, the AUC, sensitivity, specificity, PPV, and NPV were 0.918, 90.2%, 87.0%, 78.7%, and 94.4%, respectively, for F≥3, and 0.887, 92.3%, 78.3%,

54.5%, and 97.3% for F4 in HBeAg-positive patients. No significant differences were observed between the CPHBV AUCs of the HBeAg-positive and HBeAg-negative patients in predicting significant fibrosis, advanced fibrosis, and cirrhosis. (Z=0.318, 0.943, and 0.104; *P*=0.76, 0.35, and 0.92, respectively).

**Comparison of CPHBV with other noninvasive models**

We compared the AUCs among six panels for the evaluation of significant fibrosis (**Table 6, Fig. 1)**. The CPHBV model had a significantly higher AUC value for significant fibrosis in HBV-infected individuals with PNALT than APRI, FIB-4, GPR, Forn's index, and S-index (Table 6).

**DISCUSSION**

We previously demonstrated that serum CP was a potential biomarker to predict hepatic fibrosis in HBV-infected individuals [22, 23]. In this multi-center research, we further confirmed the valuable role of CP as a strong indicator to assess hepatic fibrosis in HBV-infected individuals with PNALT. In particular, our results demonstrated that serum CP had an inverse correlation with liver fibrosis. Therefore, we were able to develop the CPHBV index to predict the different stages of fibrosis (i.e., significant, advanced, and cirrhosis). Further, we validated the specificity and sensitivity of the CPHBV model and compared its prognostic value to that of previously established models. Remarkably, the newly developed CPHBV model had a significantly better predictive value for significant fibrosis than the APRI, FIB-4, and GPR scores as well as Forn's index and the S-index. To our knowledge, this is the first effort to develop a CPHBV model and validate its valuable role for assessing significant fibrosis in HBV-infected individuals with PNALT.

ALT is a valuable biomarker for the detection of hepatic damage: i.e., normal serum ALT typically indicates the absence of liver injury. However, this is not the case in chronic HBV patients with PNALT. Previous studies demonstrated that more than 30% of HBV-infected individuals with PNALT present with significant liver fibrosis [32, 33]. Indeed, our results demonstrated that 54.5% of HBV-infected individuals with PNALT had significant fibrosis, and their CP levels had an inverse correlation with hepatic fibrosis. In addition, multivariate analyses identified CP, HBsAg, and PLT as potential biomarkers that were independently correlated to liver fibrosis. In HBV-infected individuals with PNALT, analysis of the AUCs indicated that the serum CP had a reasonable diagnostic value to assess F≥2, F≥3, and F4. These results were in accordance with previous studies that suggested the potential function of CP for diagnosing fibrosis among HBV-infected individuals [22, 30]. The detection of PLT levels has repeatedly been used to predict liver fibrosis [34]. Further, our previous research demonstrated that quantitative measurement of the HBsAg level can distinguish patients with active liver injury from immune tolerant HBV-infected individuals [35]. Although the mechanisms underlying the negative correlation between the serum HBsAg and fibrosis stages remain unclear, it is reasonable to speculate that host immune responses to HBV may result in liver damage, which can lead to a reduction in the HBsAg level. Taken together, our study enabled the identification of three serum biomarkers that were negatively associated with liver fibrosis, which enabled us to develop the CPHBV model.

The CPHBV model consists of three routinely assessed and relatively inexpensive biochemical parameters (CP, HBsAg, and PLT), which can be beneficial for resource-limited institutions to accurately detect liver fibrosis. The use of the CPHBV

model will enable the accurate determination of patients in urgent need of antiviral treatment. Although this novel model was developed to diagnose significant fibrosis, it can also be used for diagnosing advanced fibrosis and cirrhosis. According to the American Association for the Study of Liver Diseases (AASLD), European Association for the Study of the Liver (EASL), Asian Pacific Association for the Study for the Liver (APASL), and World Health Organization (WHO) guidelines, it is recommended to treat patients with moderate liver necroinflammation or fibrosis without distinguishing their HBeAg status [7-9, 36]. Our CPHBV model is applicable to HBV-infected individuals with PNALT without distinguishing the HBeAg status.

Numerous novel noninvasive models to diagnose liver fibrosis have emerged. The APRI and FIB-4 scores were recommended to evaluate hepatic fibrosis by the HBV practice clinical guidelines. However, recent research showed that APRI and FIB-4 had low predictive performance in chronic HBV patients with PNALT (AUC of 0.518 and 0.597, respectively) [37]. In this study, the AUC of FIB-4 or APRI for F≥2 in HBV-infected individuals with PNALT was lower than that for the CPHBV model (all $P$<0.001). GPR is another novel predictive index for significant fibrosis in HBV-infected patents in the West African population [27]. GPR was found to be useful in our Chinese patient cohort but the predictive value of the novel CPHBV panel significantly surpassed that of GPR. Forn's index was applied to diagnose liver fibrosis in CHB individuals [38]. However, the diagnostic value of Forn's index for significant fibrosis in HBV-infected individuals with PNALT was rather limited (AUC=0.687). The S-index was specifically designed to assess significant fibrosis in CHB individuals and had superior diagnostic accuracy compared to the APRI and FIB-4[39]. Nevertheless, CPHBV was superior to the S index in our patient cohort (0.839 vs 0.722, P=0.0034). Taken together, the newly developed CPHBV

model had better performance in identifying significant fibrosis than APRI, FIB-4, GPR, Forn's index, and the S index, at least in our patient cohort.

This study had a few limitations. First, CPHBV was developed and evaluated in a multicenter cross-sectional study. Therefore, future prospective multicenter studies will be needed to verify the diagnostic value of CPHBV. Second, we used cutoff ALT values <40 IU/ml as the upper limit of normal for ALT in this study. However, in accordance with the current guidelines, ALT is estimated to be 35 IU/mL in healthy men and 25 IU/mL in healthy women [7]. Third, we used CP values tested at one time point to construct our model. Future studies should follow up with the participants to validate our results at different time points.

In conclusion, serum CP is a routinely investigated biochemical that negatively correlates with hepatic fibrosis and a potential marker to diagnose liver fibrosis in HBV-infected individuals with PNALT. The CPHBV model was found to be more efficient than the previously reported noninvasive models in diagnosing significant fibrosis among HBV-infected individuals with PNALT. The use of CPHBV might reduce the clinical need for LB in the future.

**REFERENCES**

1 **Razavi-Shearer D**, Gamkrelidze I, Nguyen MH, Chen D-S, Van Damme P, Abbas Z, Abdulla M, Abou Rached A, Adda D, Aho I, Akarca U, Hasan F, Al Lawati F, Al Naamani K, Al-Ashgar HI, Alavian SM, Alawadhi S, Albillos A, Al-Busafi SA, Aleman S, Alfaleh FZ, Aljumah AA, Anand AC, Anh NT, Arends JE, Arkkila P, Athanasakis K, Bane A, Ben-Ari Z, Berg T, Bizri AR, Blach S, Brandão Mello CE, Brandon SM, Bright B, Bruggmann P, Brunetto M, Buti M, Chan HLY, Chaudhry A, Chien R-N, Choi MS, Christensen PB, Chuang W-L, Chulanov V, Clausen MR, Colombo M, Cornberg M, Cowie B, Craxi A, Croes EA, Cuellar DA, Cunningham C, Desalegn H, Drazilova S, Duberg A-S, Egeonu SS, El-Sayed MH, Estes C, Falconer K, Ferraz MLG, Ferreira PR, Flisiak R, Frankova S, Gaeta GB, García-Samaniego J, Genov J, Gerstoft J, Goldis A, Gountas I, Gray R, Guimarães Pessôa M, Hajarizadeh B, Hatzakis A, Hézode C, Himatt SM, Hoepelman A, Hrstic I, Hui Y-TT, Husa P, Jahis R, Janjua NZ, Jarčuška P, Jaroszewicz J, Kaymakoglu S, Kershenobich D, Kondili LA, Konysbekova A, Krajden M, Kristian P, Laleman W, Lao W-cC, Layden J, Lazarus JV, Lee M-H, Liakina V, Lim Y-SS, Loo C-kK, Lukšić B, Malekzadeh R, Malu AO, Mamatkulov A, Manns M, Marinho RT, Maticic M, Mauss S, Memon MS, Mendes Correa MC, Mendez-Sanchez N, Merat S, Metwally AM, Mohamed R, Mokhbat JE, Moreno C, Mossong J, Mourad FH, Müllhaupt B, Murphy K, Musabaev E, Nawaz A, Nde HM, Negro F, Nersesov A, Nguyen VTT, Njouom R, Ntagirabiri R, Nurmatov Z, Obekpa S, Ocama P, Oguche S, Omede O, Omuemu C, Opare-Sem O, Opio CK, Örmeci N, Papatheodoridis G, Pasini K, Pimenov N, Poustchi H, Quang TD, Qureshi H, Ramji A, Razavi-Shearer K, Redae B, Reesink HW, Rios CY, Rjaskova G, Robbins S, Roberts LR, Roberts SK, Ryder SD, Safadi R, Sagalova O,

Salupere R, Sanai FM, Sanchez-Avila JF, Saraswat V, Sarrazin C, Schmelzer JD, Schréter I, Scott J, Seguin-Devaux C, Shah SR, Sharara AI, Sharma M, Shiha GE, Shin T, Sievert W, Sperl J, Stärkel P, Stedman C, Sypsa V, Tacke F, Tan SS, Tanaka J, Tomasiewicz K, Urbanek P, van der Meer AJ, Van Vlierberghe H, Vella S, Vince A, Waheed Y, Waked I, Walsh N, Weis N, Wong VW, Woodring J, Yaghi C, Yang H-I, Yang C-L, Yesmembetov K, Yosry A, Yuen M-F, Yusuf MAM, Zeuzem S, Razavi H. Global prevalence, treatment, and prevention of hepatitis B virus infection in 2016: a modelling study. *The Lancet Gastroenterology & Hepatology* 2018; **3**(6): 383-403 [DOI: 10.1016/s2468-1253(18)30056-6]

2 **Wu JF**, Chang MHJJoBS. Natural history of chronic hepatitis B virus infection from infancy to adult life -the mechanism of inflammation triggering and long-term impacts. 2015; **22**(1): 92

3 **Xiao G**, Yang J, Yan L. Comparison of diagnostic accuracy of aspartate aminotransferase to platelet ratio index and fibrosis-4 index for detecting liver fibrosis in adult patients with chronic hepatitis B virus infection: a systemic review and meta-analysis. *Hepatology* 2015; **61**(1): 292-302 [PMID: 25132233 DOI: 10.1002/hep.27382]

4 **Weemhoff JL**, Woolbright BL, Jenkins RE, Mcgill MR, Sharpe MR, Olson JC, Antoine DJ, Curry SC, Jaeschke HJLI. Plasma Biomarkers to Study Mechanisms of Liver Injury in Patients with Hypoxic Hepatitis. 2016; **37**(3)

5 **Gong Y**, Liu Z, Liao Y, Mai C, Chen T, Tang H, Tang YJN. Effectiveness of ω-3 Polyunsaturated Fatty Acids Based Lipid Emulsions for Treatment of Patients after Hepatectomy: A Prospective Clinical Trial. 2016; **8**(6): 357

6 **Kelleni MT**, Ibrahim SA, Abdelrahman AMJTM. Effect of captopril and telmisartan on methotrexate-induced hepatotoxicity in rats: impact of oxidative stress, inflammation and apoptosis.

7 **Terrault NA**, Lok ASF, McMahon BJ, Chang KM, Hwang JP, Jonas MM, Brown RS, Jr., Bzowej NH, Wong JB. Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance. *Hepatology* 2018; **67**(4): 1560-1599 [PMID: 29405329 DOI: 10.1002/hep.29800]

8 European Association for the Study of the Liver. Electronic address eee, European Association for the Study of the L. EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. *J Hepatol* 2017; **67**(2): 370-398 [PMID: 28427875 DOI: 10.1016/j.jhep.2017.03.021]

9 **Sarin SK**, Kumar M, Lau GK, Abbas Z, Chan HL, Chen CJ, Chen DS, Chen HL, Chen PJ, Chien RN, Dokmeci AK, Gane E, Hou JL, Jafri W, Jia J, Kim JH, Lai CL, Lee HC, Lim SG, Liu CJ, Locarnini S, Al Mahtab M, Mohamed R, Omata M, Park J, Piratvisuth T, Sharma BC, Sollano J, Wang FS, Wei L, Yuen MF, Zheng SS, Kao JH. Asian-Pacific

clinical practice guidelines on the management of hepatitis B: a 2015 update. *Hepatol Int* 2016; **10**(1): 1-98 [PMID: 26563120 PMCID: PMC4722087 DOI: 10.1007/s12072-015-9675-4]

10 **Rockey DC**, Caldwell SH, Goodman ZD, Nelson RC, Smith AD, American Association for the Study of Liver D. Liver biopsy. *Hepatology* 2009; **49**(3): 1017-1044 [PMID: 19243014 DOI: 10.1002/hep.22742]

11 Detlef S, Ook KYJJoCI. Evolving therapies for liver fibrosis. 2013; **123**(5): 1887-1901

12 **Udell JA**, Wang CS, Jill T, J Mark F, Ayas NT, Simel DL, Michael S, Edwin M, Yoshida EM, %J Jama. Does this patient with liver disease have cirrhosis? 2012; **307**(8): 832-842

13 **Jia J**, Hou J, Ding H, Chen G, Xie Q, Wang Y, Zeng M, Zhao J, Wang T, Hu XJJoG, Hepatology. Transient elastography compared to serum markers to predict liver fibrosis in a cohort of Chinese patients with chronic hepatitis B. 2015; **30**(4): 756-762

14 **Dong DR**, Hao MN, Li C, Peng Z, Liu X, Wang GP, Ma ALJMMR. Acoustic radiation force impulse elastography, FibroScan®, Forns' index and their combination in the assessment of liver fibrosis in patients with chronic hepatitis B, and the impact of inflammatory activity and steatosis on these diagnostic methods. 2015; **11**(6): 4174-4182

15 **Meng F**, Zheng Y, Zhang Q, Mu X, Xu X, Zhang H, Ding LJJoUiMOJotAIoUiM. Noninvasive Evaluation of Liver Fibrosis Using Real-time Tissue Elastography and Transient Elastography (FibroScan). 2015; **34**(3): 403

16 Organization WHJWHO. Guidelines for the Prevention, Care and Treatment of Persons with Chronic Hepatitis B Infection. 2015

17 **Kim WR**, Berg T, Asselah T, Flisiak R, Fung S, Gordon SC, Janssen HL, Lampertico P, Lau D, Bornstein JD, Schall RE, Dinh P, Yee LJ, Martins EB, Lim SG, Loomba R, Petersen J, Buti M, Marcellin P. Evaluation of APRI and FIB-4 scoring systems for non-invasive assessment of hepatic fibrosis in chronic hepatitis B patients. *J Hepatol* 2016; **64**(4): 773-780 [PMID: 26626497 DOI: 10.1016/j.jhep.2015.11.012]

18 **Tan YW**, Zhou XB, Ye Y, He C, Ge GHJWJoG. Diagnostic value of FIB-4, aspartate aminotransferaseto-platelet ratio index and liver stiffness measurement in hepatitis B virus-infected patients with persistently normal alanine aminotransferase. 2017; **23**(31): 5746-5754

19 **Ehrenwald E**, Chisolm GM, Fox PL. Intact human ceruloplasmin oxidatively modifies low density lipoprotein. *Journal of Clinical Investigation* 1994; **93**(4): 1493

20 **Linder MC**. Ceruloplasmin and other copper binding components of blood plasma and their functions: an update. *Metallomics* 2016; **8**(9): 887-905 [PMID: 27426697 DOI: 10.1039/c6mt00103c]

21 **Yu L**, Liou IW, Biggins SW, Yeh M, Jalikis F, Chan LN, Burkhead J. Copper Deficiency in Liver Diseases: A Case Series and Pathophysiological Considerations.

*Hepatol Commun* 2019; **3**(8): 1159-1165 [PMID: 31388635 PMCID: PMC6671688 DOI: 10.1002/hep4.1393]

22 **Zeng DW**, Liu YR, Zhang JM, Zhu YY, Lin S, You J, Li YB, Chen J, Zheng Q, Jiang JJ, Dong J. Serum ceruloplasmin levels correlate negatively with liver fibrosis in males with chronic hepatitis B: a new noninvasive model for predicting liver fibrosis in HBV-related liver disease. *PLoS One* 2013; **8**(10): e77942 [PMID: 24282481 PMCID: PMC3837017 DOI: 10.1371/journal.pone.0077942]

23 **Zeng DW**, Dong J, Jiang JJ, Zhu YY, Liu YR. Ceruloplasmin, a reliable marker of fibrosis in chronic hepatitis B virus patients with normal or minimally raised alanine aminotransferase. *World J Gastroenterol* 2016; **22**(43): 9586-9594 [PMID: 27920479 PMCID: PMC5116602 DOI: 10.3748/wjg.v22.i43.9586]

24 **Kang N-L**, Zhang J-M, Liu Y-R, Lin S, Dong J, Jiang J-J, Zhu Y-Y, Zeng D-WJC, hepatology ri, gastroenterology. Novel predictive models using serum ceruloplasmin levels for hepatic steatosis in patients with chronic hepatitis B infection. 2019

25 **Wai CT**, Greenson JK, Fontana RJ, Kalbfleisch JD, Marrero JA, Conjeevaram HS, Lok AS. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology* 2003; **38**(2): 518-526 [PMID: 12883497 DOI: 10.1053/jhep.2003.50346]

26 **Vallet-Pichard A**, Mallet V, Nalpas B, Verkarre V, Nalpas A, Dhalluin-Venier V, Fontaine H, Pol S. FIB-4: an inexpensive and accurate marker of fibrosis in HCV infection. comparison with liver biopsy and fibrotest. *Hepatology* 2007; **46**(1): 32-36 [PMID: 17567829 DOI: 10.1002/hep.21669]

27 **Lemoine M**, Shimakawa Y, Nayagam S, Khalil M, Suso P, Lloyd J, Goldin R, Njai HF, Ndow G, Taal M, Cooke G, D'Alessandro U, Vray M, Mbaye PS, Njie R, Mallet V, Thursz M. The gamma-glutamyl transpeptidase to platelet ratio (GPR) predicts significant liver fibrosis and cirrhosis in patients with chronic HBV infection in West Africa. *Gut* 2016; **65**(8): 1369-1376 [PMID: 26109530 PMCID: PMC4975834 DOI: 10.1136/gutjnl-2015-309260]

28 **Forns X**, Ampurdanes S, Llovet JM, Aponte J, Quinto L, Martinez-Bauer E, Bruguera M, Sanchez-Tapias JM, Rodes J. Identification of chronic hepatitis C patients without hepatic fibrosis by a simple predictive model. *Hepatology* 2002; **36**(4 Pt 1): 986-992 [PMID: 12297848 DOI: 10.1053/jhep.2002.36128]

29 **Zhou K**, Gao CF, Zhao YP, Liu HL, Zheng RD, Xian JC, Xu HT, Mao YM, Zeng MD, Lu LG. Simpler score of routine laboratory tests predicts liver fibrosis in patients with chronic hepatitis B. *J Gastroenterol Hepatol* 2010; **25**(9): 1569-1577 [PMID: 20796157 DOI: 10.1111/j.1440-1746.2010.06383.x]

30 **Zeng D-W**, Dong J, Jiang J-J, Zhu Y-Y, Liu Y-R. Ceruloplasmin, a reliable marker of

fibrosis in chronic hepatitis B virus patients with normal or minimally raised alanine aminotransferase. *World Journal of Gastroenterology* 2016; **22**(43): 9586 [DOI: 10.3748/wjg.v22.i43.9586]

31 European Association for the Study of the L. EASL Clinical Practice Guidelines: Management of chronic hepatitis B. *Journal of Hepatology*; **50**(2): 227-242 [DOI: 10.1016/j.jhep.2008.10.001]

32 **Andreani T**, Serfaty L, Mohand D, Dernaika S, Wendum D, Chazouillères O, Poupon RJCG, Hepatology. Chronic Hepatitis B Virus Carriers in the Immunotolerant Phase of Infection: Histologic Findings and Outcome. 2007; **5**(5): 636-641

33 **Sarin SK**, Hissar S, Pande C, Sakhuja PJG. Virologic and histologic features of chronic hepatitis B virus-infected asymptomatic patients with persistently normal ALT. 2008; **134**(5): 1376-1384

34 **Sterling RK**, Eduardo L, Nathan C, Ricard S, Mendes Cassia C, Julio M, Mark SS, Torriani FJ, Dieterich DT, Thomas DL, %J Hepatology. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. 2010; **43**(6): 1317-1325

35 **Zeng DW**, Zhang JM, Liu YR, Dong J, Jiang JJ, Zhu YY. A Retrospective Study on the Significance of Liver Biopsy and Hepatitis B Surface Antigen in Chronic Hepatitis B Infection. *Medicine (Baltimore)* 2016; **95**(8): e2503 [PMID: 26937895 PMCID: PMC4778991 DOI: 10.1097/MD.0000000000002503]

36 Organization WH. Global hepatitis report 2017: World Health Organization, 2017

37 **YW T**, XB Z, Y Y, C H, gastroenterology GGJWjo. Diagnostic value of FIB-4, aspartate aminotransferase-to-platelet ratio index and liver stiffness measurement in hepatitis B virus-infected patients with persistently normal alanine aminotransferase. 2017; **23**(31): 5746-5754

38 **Ucar F**, Sezer S, Ginis Z, Ozturk G, Albayrak A, Basar O, Ekiz F, Coban S, Yuksel O, Armutcu F, Akbal E. APRI, the FIB-4 score, and Forn's index have noninvasive diagnostic value for liver fibrosis in patients with chronic hepatitis B. *Eur J Gastroenterol Hepatol* 2013; **25**(9): 1076-1081 [PMID: 23510962 DOI: 10.1097/MEG.0b013e32835fd699]

39 **Zeng X**, Xu C, He D, Li M, Zhang H, Wu Q, Xiang D, Wang Y. Performance of several simple, noninvasive models for assessing significant liver fibrosis in patients with chronic hepatitis B. *Croatian Medical Journal* 2015; **56**(3): 272-279 [DOI: 10.3325/cmj.2015.56.272]

**Table 1. Demographic characteristics and clinical features in our patient cohort**

| | | ALL (n=275) | Training group (n=138) | Validation group (n=137) | *P*-value |
|---|---|---|---|---|---|
| Age (yr) | | 40.25 ± 9.65 | 40.90 ± 9.90 | 39.61 ± 9.37 | 0.267 |
| Gender | Male | 194 (70.5%) | 101 (73.2%) | 93 (67.9%) | 0.335 |
| | Female | 71 (29.5%) | 37 (26.8%) | 44 (32.1%) | |

| | | | | |
|---|---|---|---|---|
| CP (mg/L) | 213.01 ± 43.28 | 212.72 ± 42.68 | 213.29 ± 44.03 | 0.914 |
| Total bilirubin (μmol/L) | 13.51± 7.16 | 13.31 ± 6.46 | 13.71 ± 7.80 | 0.652 |
| Albumin (g/L) | 42.84 ± 3.68 | 42.93 ± 3.74 | 42.75 ± 3.63 | 0.692 |
| Globulin (g/L) | 28.19 ± 18.49 | 29.43 ± 6.04 | 27.01 ± 4.28 | 0.291 |
| ALT (IU/L) | 29.56 ± 9.86 | 29.65 ± 9.69 | 29.46 ± 10.07 | 0.872 |
| AST (IU/L) | 26.91 ± 7.17 | 26.71 ± 7.19 | 27.11 ± 7.16 | 0.645 |
| GGT (IU/L) | 29.48 ± 21.87 | 29.23 ± 24.34 | 29.71 ± 19.28 | 0.860 |
| TCHO (mmol/L) | 4.69 ± 0.89 | 4.61 ± 0.75 | 4.77 ± 1.00 | 0.149 |
| TG (mmol/L) | 1.15 ± 0.68 | 1.25 ± 0.80 | 1.05 ± 0.50 | 0.113 |
| CHE (IU/mL) | 8215.20 ± 2312.22 | 8409.96 ± 2358.01 | 8027.75± 2260.29 | 0.182 |
| WBC ($10^9$/L) | 5.68 ± 1.48 | 5.74 ± 1.48 | 5.62± 1.49 | 0.509 |
| PLT ($10^9$/L) | 191.96 ± 51.77 | 193.78 ± 51.91 | 190.11 ± 51.76 | 0.558 |
| HBsAg (Log IU/mL) | 3.95 ± 0.95 | 3.89 ± 0.99 | 3.40 ± 0.90 | 0.346 |
| HBV-DNA (Log IU/mL) | 5.13 ± 2.02 | 5.08± 2.01 | 5.19 ± 2.03 | 0.666 |
| PT (s) | 12.11 ± 2.31 | 12.04 ± 2.56 | 12.18 ± 2.04 | 0.626 |
| INR | 1.00± 0.19 | 0.99 ± 0.21 | 1.01 ± 0.17 | 0.662 |
| Inflammation stage, n (%) | | | | 0.942 |
| Inf 0 | 9 (2.9) | 4 (2.9) | 5 (3.6) | |
| Inf 1 | 119 (43.3) | 63 (45.7) | 56 (40.9) | |
| Inf 2 | 82 (29.8) | 40 (29.0) | 42 (30.7) | |
| Inf 3 | 43 (15.6) | 21 (15.2) | 22 (16.1) | |
| Inf 4 | 22 (8.0) | 10 (7.2) | 12 (8.8) | |

| | | | | |
|---|---|---|---|---|
| Fibrosis stage, n (%) | | | | 0.670 |
| F0 | 19 (6.9) | 12 (8.7) | 7 (5.1) | |
| F1 | 106 (38.5) | 52 (37.7) | 54 (39.4) | |
| F2 | 57 (20.7) | 26 (18.8) | 31 (22.6) | |
| F3 | 40 (14.5) | 19 (13.8) | 21 (15.3) | |
| F4 | 53 (19.3) | 29 (21.0) | 24 (17.5) | |

CP: ceruloplasmin; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: gamma glutamyl transpeptidase; TCHO: total cholesterol; TG: triglyceride; CHE: cholinesterase; WBC: white blood cell count; PLT: platelet count; HBV: hepatitis B virus; PT: prothrombin time; INR: International Normalized Ratio

**Table 2. Accuracy of CP values in diagnosing F≥2, F≥3, and F=4 as measured by AUC (n=138)**

| Steatosis degree | AUC (95% CI) | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) | Cut-off point |
|---|---|---|---|---|---|---|
| F≥2 | 0.774 (0.696-0.851) | 81.3 | 64.9 | 66.7 | 80 | ≤203.5 |
| F≥3 | 0.812 (0.732-0.892) | 82.8 | 65.9 | 83.7 | 64.4 | ≤190.5 |
| F=4 | 0.853 (0.781-0.925) | 89.3 | 65.4 | 91.7 | 58.6 | ≤182.5 |

CP: ceruloplasmin; AUC: area under the receiver operating characteristic curve

**Table 3. Clinical parameters associated with significant fibrosis in the training group (138 patients)**

| | | No significant fibrosis (n=64) | significant fibrosis (n=74) | *P*-valu e |
|---|---|---|---|---|
| Age (yr) | | 39.88 ± 9.68 | 39.61 ± 9.37 | 0.260 |
| Gender | Male | 47 (73.4%) | 54 (73.0%) | 0.951 |
| | Female | 17 (26.6%) | 20 (27.0%) | |
| CP (mg/L) | | 231.22 ± 35.26 | 196.73 ± 42.27 | <0.0001 |
| Total bilirubin (μmol/L) | | 13.11 ± 6.97 | 13.48 ± 6.05 | 0.747 |
| Albumin (g/L) | | 44.27 ± 3.29 | 41.79 ± 3.75 | <0.0001 |
| Globulin (g/L) | | 27.15 ± 5.15 | 31.35 ± 5.03 | 0.363 |
| ALT (IU/L) | | 29.25 ± 10.32 | 30.00 ± 9.17 | 0.652 |
| AST (IU/L) | | 25.77 ± 7.21 | 27.53 ± 7.11 | 0.152 |
| GGT (IU/L) | | 23.69 ± 13.96 | 33.90 ± 29.78 | 0.017 |
| TCHO (mmol/L) | | 4.82 ± 0.83 | 4.44 ± 0.62 | 0.004 |
| TG (mmol/L) | | 1.31 ± 0.74 | 1.21 ± 0.85 | 0.474 |
| CHE (IU/mL) | | 9254.48 ± 2583.76 | 7710.21 ± 1903.41 | <0.0001 |
| WBC ($10^9$/L) | | 5.78 ± 1.36 | 5.71 ± 1.59 | 0.766 |
| PLT ($10^9$/L) | | 212.63 ± 50.24 | 177.49 ± 47.94 | <0.0001 |

| | | | |
|---|---|---|---|
| HBsAg (Log IU/mL) | 4.40 ± 0.78 | 3.45 ± 0.96 | <0.0001 |
| HBV-DNA (Log IU/mL) | 5.17± 2.33 | 5.00 ± 1.70 | 0.614 |
| PT (s) | 11.71 ± 2.81 | 12.33 ± 2.31 | 0.161 |
| INR | 0.96 ± 0.23 | 1.02 ± 0.19 | 0.103 |

CP: ceruloplasmin; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: gamma glutamyl transpeptidase; TCHO: total cholesterol; TG: triglyceride; CHE: cholinesterase; WBC: white blood cell count; PLT: platelet count; HBV: hepatitis B virus; PT: prothrombin time; INR: International Normalized Ratio.

**Table 4. Diagnostic value of CPHBV in the training and validation groups**

| **Variable** | **Training group (n=138)** | | | **Validation group (n=137)** | | |
|---|---|---|---|---|---|---|
| | **F≥2** | **F≥3** | **F=4** | **F≥2** | **F≥3** | **F=4** |
| AUC | 0.842 | 0.920 | 0.904 | 0.805 | 0.886 | 0.863 |
| 95%CI | 0.777-0.907 | 0.872-0.967 | 0.842-0.955 | 0.733-0.877 | 0.827-0.946 | 0.784-0.943 |
| Cut-off | 0.0304 | 0.496 | 0.553 | 0.174 | 0.176 | 0.206 |
| Sensitivity % | 81.1 | 90.9 | 96.2 | 70.3 | 91.1 | 95.8 |
| Specificity | 71.9 | 79.6 | 71.4 | 80.3 | 77.2 | 70.8 |
| Youden's index | 0.530 | 0.705 | 0.676 | 0.536 | 0.683 | 0.666 |
| PPV | 76.9 | 67.8 | 43.9 | 82.1 | 66.1 | 41.1 |
| NPV | 76.7 | 94.9 | 98.8 | 71.0 | 94.7 | 98.7 |

AUC: area under the receiver operating characteristic curve; NPV: negative predictive value; PPV: positive predictive value

**Table 5. Diagnostic value of CPHBV in HBeAg-positive and HBeAg-negative patients**

| Variable | HBeAg positive (n=118) | | | HBeAg negative (n=157) | | |
|---|---|---|---|---|---|---|
| | F≥2 | F≥3 | F=4 | F≥2 | F≥3 | F=4 |
| AUC | 0.829 | 0.918 | 0.887 | 0.813 | 0.880 | 0.882 |
| 95%CI | 0.754-0.904 | 0.861-0.976 | 0.814-0.960 | 0.747-0.878 | 0.826-0.935 | 0.821-0.944 |
| Cut-off | 0.1697 | 0.5197 | 0.5264 | 0.8749 | 0.8810 | 0.8833 |
| Sensitivity % | 74.2 | 90.2 | 92.3 | 57.5 | 77.1 | 87.5 |
| Specificity | 83.2 | 87.0 | 78.3 | 89.9 | 83.2 | 74.2 |
| Youden's index | 0.581 | 0.773 | 0.706 | 0.473 | 0.603 | 0.617 |
| PPV | 83.6 | 78.7 | 54.5 | 87.7 | 67.3 | 38.2 |
| NPV | 74.6 | 94.4 | 97.3 | 62.6 | 89.0 | 97.0 |

AUC: area under the receiver operating characteristic curve; NPV: negative predictive value; PPV: positive predictive value

**Table 6. Comparisons of CPHBV with other predictive models for assessing F≥2 (n=275)**

| Model | AUC (95% CI) | Youden index | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) | *P*-value |
|---|---|---|---|---|---|---|---|
| CPHBV | 0.839 (0.792-0.886) | 0.512 | 80.1 | 71.1 | 76.2 | 72.4 | |
| APRI | 0.704 (0.639-0.768) | 0.355 | 75.9 | 59.6 | 68.5 | 66.1 | <0.001 |
| FIB-4 | 0.678 (0.612-0.745) | 0.330 | 71.9 | 61.4 | 68.8 | 64.2 | <0.001 |
| GPR | 0.704 (0.640-0.768) | 0.350 | 74.5 | 60.5 | 70.1 | 66.7 | <0.001 |
| Forn's index | 0.687 (0.622-0.753) | 0.359 | 63.1 | 72.8 | 74.2 | 61.5 | <0.001 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| S-index | 0.722 (0.659-0.784) | 0.357 | 78.7 | 57.0 | 69.9 | 69.7 | 0.0034 |

AUC: area under the receiver operating characteristic curve; NPV: negative predictive value; PPV: positive predictive value

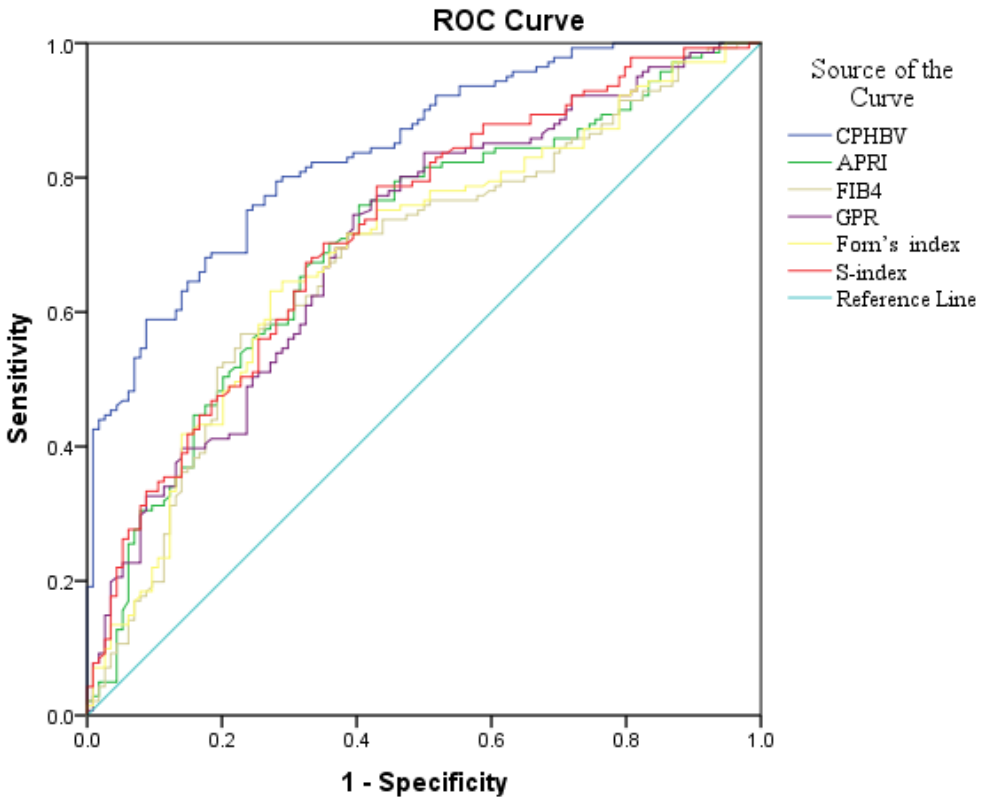



**Figure 1.** ROC curves for the noninvasive models (CPHBV, APRI, FIB-4, GPR, Forn's index, S-index) in all the study subjects.