Diagnostic Accuracy Study

Medicine

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Clinical significance of serum Ck18-M65 and M30 levels in patients with chronic hepatitis B combined with nonalcoholic steatohepatitis and liver fibrosis

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Abstract

In this study, we aimed to explore the clinical significance of serum CK18-M65 and CK18-M30 levels in patients with chronic hepatitis B (CHB) complicated by nonalcoholic steatohepatitis (NASH) and liver fibrosis. The observation and control groups comprised 133 patients with CHB complicated by NASH and 50 healthy patients from our hospital, respectively. Liver function indices, including alanine aminotransferase, glutamic aminotransferase, γ-glutamyltransferase, total bilirubin, total protein, and total cholesterol, were determined using an automatic biochemical analyzer. Hyaluronic acid, type III procollagen, type IV collagen, laminin, and CK18-M65 and M30 levels were detected using ELISA. Serum CK18-M65 and M30 levels in patients with CHB complicated by NASH were positively correlated with the liver fibrosis stage ($P < .05$). While serum CK18-M65 demonstrated a low diagnostic value for liver fibrosis in the S0-1 stage, it exhibited good diagnostic value for S2-3 stage liver fibrosis. Serum CK18-M30 displayed good diagnostic value for S0-1 and S2-3 hepatic fibrosis, particularly for S2-3 hepatic fibrosis. Elevated serum CK18-M65 and CK18-M30 levels in patients with CHB complicated with NASH suggest their potential utility in evaluating the progression of liver fibrosis in this population. In particular, CK18-M30 exhibits superior diagnostic efficiency.

Abbreviations: ALT = alanine aminotransferase, AUC = area under a receiver operating characteristic curve, CHB = chronic hepatitis B, CHOL = cholesterol, CK18 = cytokeratin 18, GOT/AST = glutamate aminotransferase, HA = hyaluronic acid, IV-C = type IV collagen, LN = laminin, NAFLD = nonalcoholic fatty liver disease, NASH = nonalcoholic steatohepatitis, PIIIP = type III pre-collagen, ROC = receiver operating characteristic, TBIL = total bilirubin, TP = total protein, γ-GT = γ-glutamyltranspeptidase.

Keywords: chronic hepatitis B, CK18-M30, CK18-M65, liver fibrosis, nonalcoholic steatohepatitis

1. Introduction

Chronic hepatitis B (CHB) is a viral infection that damages the liver and can cause acute or chronic disease.[1] Nonalcoholic fatty liver disease (NAFLD) is characterized by fat accumulation in liver parenchymal cells in the absence of excessive alcohol consumption.[2,3] Patients with CHB face an increased risk of developing NAFLD, making patients with combined CHB and NAFLD an important target for research.[4] NAFLD also increases the likelihood of liver fibrosis, and the coexistence of CHB and NAFLD can exacerbate liver damage and accelerate liver fibrosis. Despite the prevalence of CHB and NAFLD, there is insufficient knowledge regarding risk markers and predictors of liver fibrosis for early detection and management.

The most accurate test for liver fibrosis is liver biopsy for pathological diagnosis, which can determine the disease severity and patient risk grading.[5] However, liver biopsy is invasive, can be traumatic and high-risk, making it poorly accepted by patients.[6] Thus, finding an ideal noninvasive method for assessing liver fibrosis is clinically important. As a noninvasive test, serum indices are safe, economical, and reproducible, and are widely used in the diagnosis and treatment of various diseases.[7] Serum liver fiber 4 levels have been widely used as a sensitive indicator of liver fibrosis, and their elevated levels reflect the continuous progression of liver fibrosis.[8] However, studies have suggested that the progression of liver fibrosis to cirrhosis may decrease liver fiber 4 levels, and that the use of serum liver fiber

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

The authors have no funding and conflicts of interest to disclose.

This paper has been reviewed by relevant departments of our hospital, such as the Science and Education Department, Medical Department and Ethics Committee of Shenzhen Hospital, Peking University. The research content involved in this research meets the requirements of medical ethics and academic morality of our hospital, and the research content is reasonable, the risks are controllable, and there are no violations. The relevant research carried out is in line with the safe, standardized and true scientific research guiding principles, and in line with the requirements of the clinical research ethics code.

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How to cite this article: Dai L, Yan Y, Chen Q. Clinical significance of serum Ck18-M65 and M30 levels in patients with chronic hepatitis B combined with nonalcoholic steatohepatitis and liver fibrosis. Medicine 2024;103:23(e38342).

Received: 12 September 2023 / Received in final form: 27 April 2024 / Accepted: 2 May 2024

http://dx.doi.org/10.1097/MD.0000000000038342

4 items alone as a diagnostic method for the degree of liver fibrosis may pose a risk of misdiagnosis.[7,8]

The mature liver cytoskeleton is primarily composed of cytokeratin 18 (CK18).[9] When hepatocyte apoptosis is triggered by infection, alcohol, or other factors, CK18 decomposes into different fragments, including antigenic fragments containing M65 and M30. It is assumed that serum CK18-M65 and M30 levels can better reflect the degree of hepatic fibrosis.[10,11] Liver function indices are important indicators for the clinical assessment of liver tissue conditions and effectively reflect hepatocyte damage. In this study, patients with CHB and NAFLD were tested for serum CK18-M65 and M30, liver fiber 4 items, and liver function to investigate the clinical significance of CK18-M65 and M30 in the assessment of the degree of hepatic fibrosis and provide a research basis for diagnosing hepatic fibrosis in these patients.

2. Materials and methods

2.1. General information

One hundred and thirty-three patients with CHB and NAFLD who visited our hospital between February 2021 and December 2022 were included in this study. Among them, 87 were male and 46 were female, aged between 20 and 68 years old, with a mean age of 41.56 ± 14.78 years.

The inclusion criteria were as follows: positive hepatitis B surface antigen for more than 6 consecutive months; hepatitis B virus gene ≥ 105 IU/mL; alanine aminotransferase (ALT) levels 1 to 2 times higher than the normal range (6–24 U/L); diagnosis of NAFLD based on the 2018 Chinese Medical Association Guidelines for the Prevention and Treatment of NAFLD; informed consent was provided for hepatic puncture biopsy; and approval from the hospital's ethics committee.

The exclusion criteria were as follows: hepatitis C virus, hepatitis A virus, hepatitis D virus, and other hepatophilic viruses; liver damage due to alcohol, drugs, immunity, or other causes; presence of diabetes, hypertension, tumors, and heart, kidney, lung, and other organ diseases; recent antiviral drug treatment; acute hepatitis B and liver failure, cirrhosis patients; patients with acute hepatitis B, liver failure, or cirrhosis; and secondary factors causing liver fat deposition, such as heavy alcohol consumption, long-term use of drugs causing liver steatosis or monogenic genetic diseases. Fifty healthy patients without a family history of hepatitis B and with normal physical examination results were selected as the control group.

According to the liver pathological puncture results, patients were grouped according to hepatic fibrosis stage: S0 stage (14 males, 8 females, mean age [36.56 ± 12.37]), S1 stage (24 males, 14 females, mean age [38.59 ± 17.61]), S2 stage (29 males, 12 females, mean age [41.66 ± 11.14]), S3 stage (15 males, 10 females, mean age [42.66 ± 11.14]), and S4 stage (2 males, 1 female, mean age [45.31 ± 12.50]). An additional, 50 healthy people who underwent physical examination in our hospital during the same period were selected as the control group (31 men, 19 women, mean age [32.68 ± 15.49] years).

No statistically significant differences in age or sex were observed between the groups, and they were comparable. This study was approved by the Ethics Committee of our hospital and informed consent was obtained from all patients.

2.2. Research methods

2.2.1. Serum biochemical index testing. On the day of the liver biopsy, 5 mL of fasting venous blood was collected, and liver function indexes were detected using a fully automatic biochemistry instrument. The detection items included ALT, glutamate aminotransferase (AST), total bilirubin (TBIL), γ-glutamyltranspeptidase (γ-GT), total protein (TP), and total cholesterol (CHOL). Serum samples were transferred to clean Epp tubes and stored at −80 °C. Additionally, hyaluronic acid (HA), type III pre-collagen (PIIIP), type IV collagen (IV-C), laminin (LN), CK18-M65, and CK18-M30 were measured by ELISA. The kits were purchased from Jiangsu Enzyme Immunity Industry Co., and each batch of experiments included positive and blank controls.

2.2.2. Liver biopsy and liver fibrosis stage. Patients underwent ultrasonic localization liver puncture biopsy and were anesthetized prior to surgical commencement. After the location of the puncture point was determined by ultrasound examination, a disposable 16G puncture needle was used to obtain liver tissue >2 cm in size via percutaneous puncture. The liver tissue samples were immediately fixed in 10% formalin, embedded in paraffin, and double-stained with hematoxylin and eosin HE and Masson trichrome. Liver fibrosis severity was graded as follows: S0, no fibrosis observed on biopsy; S1, enlarged hepatic portal bundles without interval formation; S2, enlarged hepatic portal bundles with small interval formation; S3, more intervals without cirrhosis; and S4, cirrhosis.

2.3. Statistical processing

Data were analyzed using IBM SPSS Statistics version 22.0 (IBM Corp., Armonk NY), and measurement data were expressed as x ± s. *t* Tests were used for inter-group comparisons. Receiver operating characteristic (ROC) curves were used to analyze the diagnostic value of serum CK18-M65 and CK18-M30 for liver fibrosis. Statistical significance was set at $P < .05$.

Table 1

Comparison of biochemical indexes in patients with CHB and NAFLD liver fibrosis of different stages.

Liver fibrosis classification	Control (n = 50)	S0 (n = 22)	S1 (n = 38)	S2 (n = 41)	S3 (n = 25)	S4 (n = 7)	*F*	*P*
ALT (U/L)	18.74 ± 9.83	51.74 ± 9.83	60.93 ± 10.21	80.28 ± 12.52	88.38 ± 14.78	96.7 ± 17.74	15.348	.045
AST (U/L)	16.93 ± 9.82	38.93 ± 9.82	45.39 ± 13.80	51.95 ± 13.07	62.78 ± 15.03	70.8 ± 19.68	12.459	.021
TBIL (μmol/L)	14.56 ± 5.49	25.38 ± 5.49	29.84 ± 6.75	32.84 ± 7.03	38.39 ± 7.82	41.34 ± 8.72	95.567	.075
TP (g/L)	72.67 ± 8.84	48.42 ± 8.84	41.38 ± 5.12	34.28 ± 5.72	33.92 ± 4.20	33.48 ± 3.89	68.754	.091
γ-GT (U/L)	28.75 ± 5.29	31.94 ± 6.32	42.86 ± 7.81	46.34 ± 8.45	58.82 ± 10.76	72.34 ± 12.05	72.894	.026
CHOL (mmol/L)	3.19 ± 1.28	4.53 ± 1.28	5.39 ± 1.51	6.15 ± 2.01	6.78 ± 2.13	7.81 ± 2.56	98.258	.065
HA (ng/mL)	49.38 ± 8.67	68.38 ± 12.34	82.84 ± 14.95	103.84 ± 19.89	148.39 ± 30.32	261.34 ± 43.57	119.225	.038
PIIIP (ng/mL)	14.42 ± 12.36	18.42 ± 5.79	34.38 ± 8.61	49.28 ± 9.29	68.92 ± 11.50	73.42 ± 19.33	48.724	.042
IV-C (ng/mL)	67.89 ± 9.28	84.94 ± 12.25	112.86 ± 21.98	156.34 ± 22.45	186.82 ± 30.86	242.34 ± 48.24	77.894	.011
LN (ng/mL)	102.75 ± 5.75	149.93 ± 9,67	155.39 ± 11.15	161.95 ± 12.62	182.78 ± 15.13	201.86 ± 20.58	101.459	.027
CK18-M65 (ng/mL)	227.74 ± 46.67	419.38 ± 58.04	568.84 ± 83.93	632.84 ± 92.46	848.39 ± 120.82	961.32 ± 193.57	118.254	.043
CK18-M30 (ng/mL)	95.42 ± 19.56	158.42 ± 40.84	218.38 ± 54.12	394.28 ± 62.72	621.92 ± 81.20	703.4 ± 99.77	94.345	.007

ALT = alanine aminotransferase, AST = glutamate aminotransferase, CHB = chronic hepatitis B, CHOL = cholesterol, HA = hyaluronic acid, IV-C = type IV collagen, LN = laminin, NAFLD = nonalcoholic fatty liver disease, PIIIP = type III pre-collagen, TBIL = total bilirubin, TP = total protein, γ-GT = γ-glutamyltranspeptidase.

3. Results

3.1. Comparison of biochemical indexes in patients with different stages of liver fibrosis

The general data and biochemical indices of patients with different stages of CHB and NAFLD liver fibrosis are presented in Table 1. There were no statistically significant differences in TP, TBIL, and CHOL among patients with S0, S1, S2, S3, and S4 stages ($P > .05$). In contrast, significant differences were observed in ALT, AST, γ-GT, HA, PIIIP, IV-C, LN, CK18-M65, and CK18-M30 ($P < .05$).

3.2. Diagnostic value of serum CK18-M65 and CK18-M30 on the degree of liver fibrosis

Patients with CHB and NAFLD were divided into 2 groups: the mild and moderate liver fibrosis group (S0-1 stage) and the severe fibrosis group (S2-3 stage); S4 stage was excluded due to its association with cirrhosis and small sample size. The diagnostic value of CK18-M65 and M30 on the degree of liver fibrosis is shown in Figure 1 and Tables 2–4. ROC curve analysis revealed that serum CK18-M65 level had a lower diagnostic value for S0-1 stage liver fibrosis but exhibited better diagnostic accuracy for S2-3 stage liver fibrosis. Serum CK18-M30 and serum CK18-M30/CK18-M65 demonstrated superior diagnostic value for both S0-1 and S2-3 stage liver fibrosis, with particularly robust efficacy in S2-3 stage fibrosis.

4. Discussion

CHB combined with NAFLD poses a significant public health challenge, with fibrosis being the most important pathological feature in chronic liver disease.[12,13] The progression of liver fibrosis and organ failure are common outcomes in many chronic liver diseases; early diagnosis and treatment

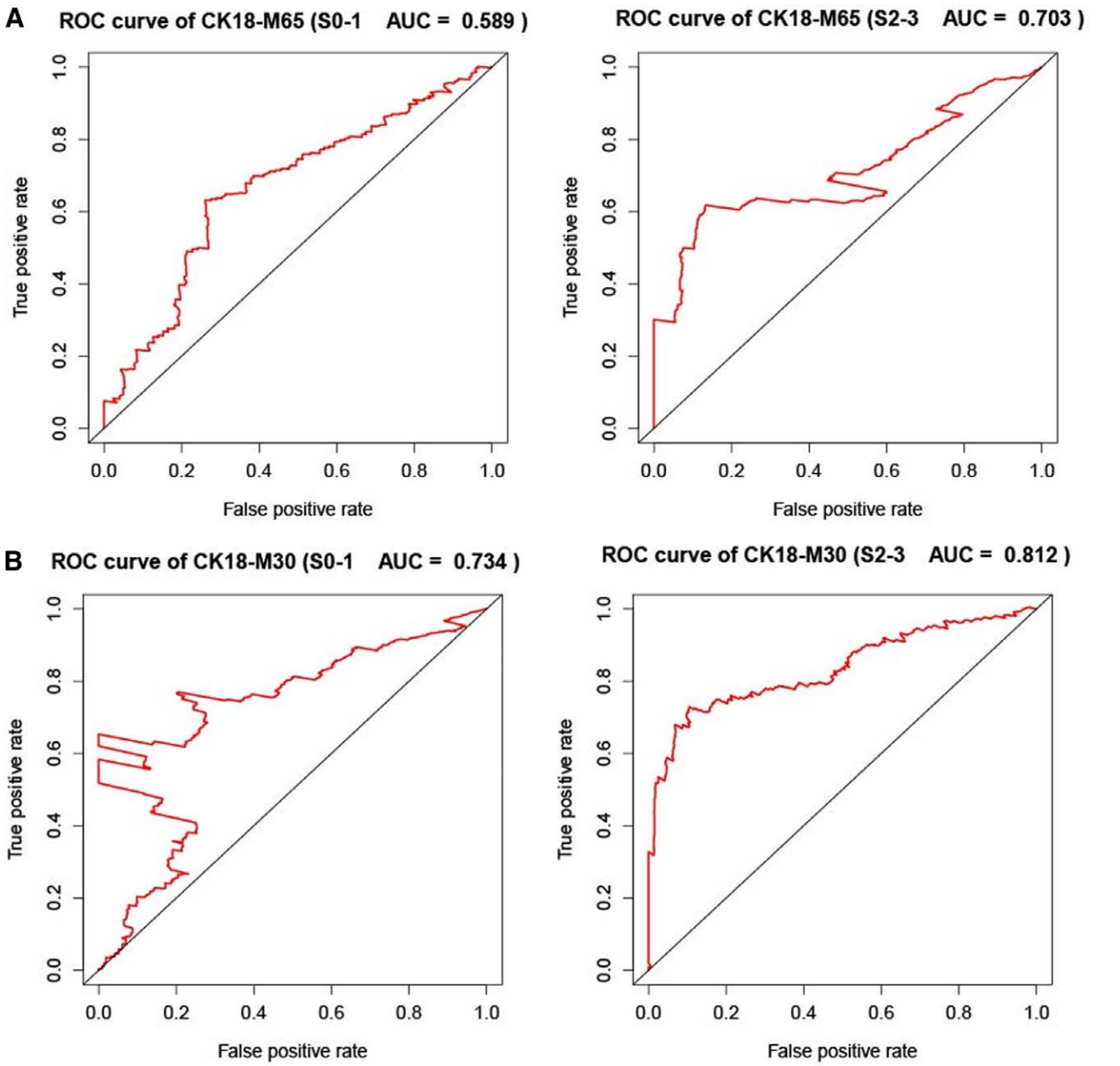

Figure 1. ROC curve of CK18-M65 and CK18-M30 accessing the prognostic model on the degree of liver fibrosis in patients with CHB and NAFLD. (A) ROC curve of CK18-M65 (B) ROC curve of CK18-M30. CHB = chronic hepatitis B, NAFLD = nonalcoholic fatty liver disease, ROC = receiver operating characteristic.

Table 2

Diagnostic value of serum CK18-M65 on the degree of liver fibrosis in patients with CHB and NAFLD.

Indicators	AUC	95% CI	Threshold value	Sensitivity (%)	Specificity (%)
S0-1	0.589	0.467–0.698	537.98	66.42	63.34
S2-3	0.703	0.513–0.742	841.84	69.39	74.95

AUC = area under a receiver operating characteristic curve, CHB = chronic hepatitis B, NAFLD = nonalcoholic fatty liver disease.

Table 3

Diagnostic value of serum CK18-M30 on the degree of liver fibrosis in patients with CHB and NAFLD.

Indicators	AUC	95% CI	Threshold value	Sensitivity (%)	Specificity (%)
S0-1	0.734	0.685–0.752	213.84	71.55	77.43
S2-3	0.812	0.632–0.897	587.87	82.74	78.58

AUC = area under a receiver operating characteristic curve, CHB = chronic hepatitis B, NAFLD = nonalcoholic fatty liver disease.

Table 4

Diagnostic value of serum CK18-M30/CK18-M65 on the degree of liver fibrosis in patients with CHB and NAFLD.

Indicators	AUC	95% CI	Threshold value	Sensitivity (%)	Specificity (%)
S0-1	0.786	0.685–0.886	205.73	74.77	79.54
S2-3	0.836	0.794–0.878	483.49	84.94	79.68

AUC = area under a receiver operating characteristic curve, CHB = chronic hepatitis B, CI = confidence interval, NAFLD = nonalcoholic fatty liver disease.

can significantly prolong patient survival.[14] Clinical assessments of liver condition in patients with CHB or NAFLD rely on indicators, including ALT, AST, TBIL, TP, γ-GT, and CHOL.[15] Additionally, HA, PIIIP, IV-C, and LN have been shown to be sensitive indicators of liver fibrosis.[16] While HA has a certain utility in diagnosis early liver fibrosis, PIIIP, IV-C, and LN lack distinctive diagnostic value for early liver fibrosis. Consequently, there is an urgent need to identify new markers.[17–19]

Recent studies exploring novel liver fibrosis markers, such as CK18, microRNA122, and tumor necrosis factor receptor, have opened new avenues for the early diagnosis of hepatic fibrosis.[20,21] Among these markers, CK18-M30 has garnered significant attention in liver disease studies. Several of which have shown that the serum level of the CK18-M30 fragment can more accurately reflect pathological changes in the liver as well as the degree of liver fibrosis.[22–24] One study found that 2 biomarkers, CK18-M30 and M65, could effectively distinguish NAFLD patients with different fibrosis stages from healthy controls.[25,26] Consistent with previous findings, our study underscores the elevated expression of CK18-M65 and M30 in patients with CHB and NAFLD and reveals statistically significant differences in serum levels in patients with different degrees of liver fibrosis, suggesting that CK18-M65 and M30 are associated with the degree of liver fibrosis.

The progression of NAFLD is driven by inflammatory mechanisms involving apoptosis and necrosis of hepatocytes, which ultimately result in the development of liver fibrosis and cirrhosis.[23] M30 levels indicate apoptosis, and M65 levels indicate both apoptosis and necrosis.[25] ROC analysis further supports this correlation, with the area under a ROC curve (AUC) of CK18-M65 for diagnosing S0-1 hepatic fibrosis at 0.589, and for stage S2-3 hepatic fibrosis at 0.703; AUC of CK18-M30 for diagnosing S0-1 hepatic fibrosis at 0.734, and for stage S2-3 hepatic fibrosis at 0.812. Notably, CK18-M30/CK18-M65 exhibits superior diagnostic performance, with an AUC of 0.786 for S0-1 stage and 0.836 for S2-3 stage liver fibrosis. These results emphasize the potential of CK18-M30 as a robust indicator for assessing liver fibrosis.

5. Limitation

This study has some limitations, primarily attributable to the limited sample size and the short follow-up duration, and long-term effects were not explored. Future investigations should focus on the mechanisms of CK18-M30 and CK18-M65 and the development of NAFLD in patients with CHB, improve clinical tests for predicting the degree of liver fibrosis in patients with chronic liver disease, and conduct in-depth research at the molecular biology level to guide prevention and treatment in the clinic.

6. Conclusion

In summary, patients with CHB and concurrent NAFLD exhibit heightened levels of serum CK18-M30 and CK18-M65. CK18-M30 can effectively reflect the degree of hepatic fibrosis in patients with CHB combined with NAFLD. Simultaneous monitoring of CK18-M30 and CK18-M65 levels will help to determine the degree of liver fibrosis.

Acknowledgments

The authors like to thank Editage (www.editage.cn) for English language editing.

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