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Serum GP73 combined AST and GGT reflects moderate to severe liver inflammation in chronic hepatitis B

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ABSTRACT

Background: Non-invasive method to identify chronic hepatitis B (CHB) patients with at least moderate inflammatory lesion but no increased ALT is an unmet need.

We evaluated Golgi protein 73 (GP73) based hepatic inflammation model (HIM) as a serum surrogate for liver necroinflammatory activity, especially in those with ALT less than traditional upper concentration.

Methods: The diagnostic performances of HIM were evaluated in 2 independent cohorts of liver biopsy proved CHB patients by receiver operating characteristic (ROC) curve analysis.

Results: HIM, a suggested index combined GP73, gamma-glutamyltransferase and AST, could significantly improve the diagnostic accuracy for liver necroinflammation (Area under ROC, AUROC: 0.890). More importantly, HIM still exhibit excellent diagnostic value (AUROC: 0.873) to identify patients with at least moderate liver necroinflammatory activity but with ALT < 40 U/l. Serum GP73 was the major source of improvement for diagnostic model's accuracy.

Conclusion: HIM is probably a promising non-invasive index to identify CHB patients with moderate to severe liver necroinflammation, especially in those with ALT < 40 U/l.

1. Introduction

Chronic hepatitis B virus (HBV) infection is a worldwide health problem, which can lead to liver fibrosis, cirrhosis, liver failure and hepatocellular carcinoma (HCC) [1]. Longitudinal studies indicated that patients with liver necroinflammatory activation and/or fibrosis are at increased risk of end stage liver diseases including HCC. Fortunately, timely antiviral treatment could slow down or even reverse the progression of chronic liver disease and prevent occurrence of end-stage liver disease [2–4]. Thus, liver necroinflammation and/or fibrosis have been recommended critical indications for timely antiviral treatment [5,6], Currently, aberrant elevation of alanine transaminase (ALT) activity is an important laboratory indication to initiate antiviral treatment. However, as previously reported, about 13.8% to 47.5% HBsAg positive individuals underwent constantly liver damage but with normal ALT [7–11]. Though histological evaluation is a “golden standard” for liver damage diagnosis, only people who meet the standards were recommended for liver biopsy [12]. Therefore, additional non-invasive biomarkers are needed to identify individuals who have active liver necroinflammation among those chronic HBV infected population.

GP73 is a 73 kD protein located in Golgi membrane, which can be sliced by proprotein convertase furin to release into blood. Increased serum GP73 has been suggested as a surrogate biomarker for liver fibrosis and cirrhosis [13–16]. Increased GP73 was also observed in liver inflammation, which is the major cause of liver fibrosis [17–19]. Therefore, it is worthwhile to explore the diagnostic potential of GP73 based diagnostic model for liver necroinflammatory lesion in patients with chronic hepatitis B.

Abbreviations: GP73, Golgi protein 73; ROC, receiver operating characteristic curve; HB, chronic hepatitis B; HBsAg, hepatitis B surface antigen; TBA, total bile acid; ALB, albumin; PA, prealbumin

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In this study, several GP73 based models were established to optimize the diagnostic accuracy for moderate liver necroinflammation. Among these models, the model which combined GP73, aspartate aminotransferase (AST) and gamma glutamyltransferase (GGT) was defined as the hepatic inflammation model (HIM) to reflect the presence of moderate liver necroinflammation. Then, serum GP73, the major source of improvement for diagnostic model's accuracy, has been further explored in outpatients with chronic hepatitis B infection, and its relationship between liver necroinflammation was then confirmed from various aspects.

2. Subjects and methods

2.1. Clinical characteristics of patients

This study included two independent cohorts. The first retrospective cohort was composed of the outpatients from 2010 to 2014 from 302 Military Hospital, Beijing, China. All patients had laboratory evidences of chronic HBV infection (HBsAg and/or HBV DNA positive for at least 6 months) and had stored serum available. None of them received antiviral treatment in the past 6 months. All of participants had accepted liver biopsy to verify liver disease progression. Following indexes including total Protein (TP), albumin (Alb),prothrombin time (PT), platelet (PLT), ALT, AST, alkaline phosphatase (AKP), GGT, total bilirubin (TBIL), HBsAg, HBV DNA, anti-HBs, HBeAg, anti-HBe and anti-HBc were collected for further analysis. Patients who fell under any of the following criteria were excluded: hepatocellular carcinoma, non-alcoholic fatty liver disease, autoimmune liver disease, drug induced liver disease, metabolic or genetic disease and alcohol consuming. Also, patients with solid clinical evidences, or imagine changes for liver cirrhosis were excluded.

The second cohort was part of patients from the study cohort previously reported [20]. Patients in this cohort all accompanied with significant liver fibrosis, and a small group of them had accepted liver biopsies both at the initiation of treatment and 78 weeks post Entecavir treatment. Serum specimens were collected at baseline and every 26 weeks thereafter to monitor changes of serum markers and other clinical variables. The normal healthy control has been previously described [21].

This study was approved by the Ethics Committee of 302 Military Hospital of China and Beijing Friendship Hospital. Informed consent was obtained from all participants. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the Helsinki declaration. Studies were registered with the ClinicalTrials.gov. This study was approved by the Ethics Committee of 302 Military Hospital of China and Beijing Friendship Hospital. The Informed consent was obtained from all participants. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the Helsinki declaration. All the animal experiments performed in this study were approved by the animal ethics committee of Peking University Health Science Center and operated in accordance with relevant guidelines and regulations.

2.2. Liver histology

The specimens of liver biopsy were stained with Hematoxylin-eosin (HE) and Masson. Liver necroinflammatory activity and liver fibrosis were scored on 0–3 grade and 0–4 grade separately according to METAVIR scoring system [22]. In brief, necroinflammatory activity was calculate by piecemeal necrosis and lobular necrosis. A0 = no piecemeal necrosis with no or mild lobular necrosis; A1 = mild piecemeal necrosis with none or moderate lobular necrosis, or no piecemeal necrosis with moderate lobular necrosis. A2 = no or mild piecemeal necrosis with severe lobular necrosis, or moderate piecemeal necrosis with mild or moderate lobular necrosis. A3 = moderate piecemeal necrosis with severe lobular necrosis, or severe piecemeal necrosis. ≥F2 was defined as significant fibrosis, ≥F3 was defined as advanced fibrosis and ≥A2 was defined as moderate liver necroinflammatory activity. All histology of biopsies were evaluated by two pathologists independently blinded to clinical data.

2.3. Serum GP73 measurement and biochemical indicators

Serum GP73 concentration was determined with a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) kit (Hotgen Biotech Inc., Beijing, China), according to the manufacturer's protocol. Measurement of ALT, AST, AKP and GGT were performed on Backman Automatic biochemical measurement system LX20 (Beckman). TP, Alb, TBIL, prothrombin time (PT), PLT, HBsAg, HBV DNA, anti-HBs, HBeAg, anti-HBe and anti-HBc were performed on.

2.4. Statistic analysis

The data were analyzed with IBM SPSS Statistic 22.0 software and performed with GraphPad Prism version 5.0. Patients characteristics were described as mean ± SEM or median (-IQR, +IQR) depending on distribution of data. The difference between sample groups was tested using ANOVA following rank transformation or *t*-test determined by distribution of data. The Spearman's rank correlation coefficient test was used to describe the association between 2 variables.

Binary logistic regression models were used to calculate the combined diagnostic model for liver necroinflammation and performed on SPSS. In this study, stepwise regression was used to select the significant predictors for liver necroinflammation and the predictive probabilities ($0 > p > 1$) was calculated based on formula:

$$P = \frac{1}{1 + e^{-(a+b_1x_1+b_2x_2+b_3x_3+\cdots+b_nx_n)}}$$

where a is constant, bn is Unstandardized Coefficients, x is significant predictive indicator.

The receiver-operating characteristic (ROC) curve analysis with a 95% confidence interval (CI) was conducted by using Medcalc (15.6.1) to evaluate the diagnostic value of regression model. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated by Medcalc. Optimal cut-offs for AST and combined predict model were selected to maximize the Youden index. For GP73 and ALT, predefined cut-offs were used (> 86 ng/ml for GP73 to distinguish no or mild liver necroinflammation and at least moderate liver necroinflammation. For ALT, 40 U/l the generally accepted upper limitation of normal was used in this study. All tests of significance were 2-tailed and $p < .05$ was considered statistically significant.

3. Results

3.1. Clinical characteristics of the patients

As shown in Table 1, the 302 Military Hospital of China cohort composed of 367 treatment naïve individuals with laboratory evidence of chronic hepatitis B infection. In addition, 82 patients with significant liver fibrosis containing 39 longitudinal patients from the Friendship hospital and 121 healthy volunteers were enrolled.

3.2. Construction of novel models for the assessment of liver necroinflammation

By using Spearman test, the correlation between necroinflammatory activity and common clinical indexes was explored (Table 2). And subsequent multivariate analysis revealed that, through binary logistic regression, from 12 indicators, only ALT, AST, GP73 and AKP were significant biomarkers for moderate liver necroinflammation.

Table 1
Clinical characteristics of all individual.

Variable	Beijing 302 hospital cohort	Friendship hospital cohort
Number	367	82
Sex (male/female)	230/137	68/14
Age (y)	30.0 (23.0, 39.0)	39.0 (31.0, 45.0)
PLT	182.0 (146.5, 218.5)	
TP	67.0 (63.0, 70.0)	
ALB	40.0 (37.0, 42.0)	42.2 (39.2, 45.1)
PT	11.7 (11.0, 12.3)	12.7 (12.1, 13.9)
ALT (U/l)	58.0 (31.0, 114.0)	83.5 (51.1, 139.8)
AST (U/l)	41.0 (27.0, 78.0)	52.0 (38.7, 89.9)
GGT	31.0 (17.0, 59.0)	
AKP	79.0 (62.0, 104.0)	
GP73	86.4 (61.8, 123.4)	
TBIL	12.0 (8.8, 17.1)	14.6 (11.4, 20.0)
TBA	8.0 (4.0, 14.0)	
HBeAg (+/−)	264/103	65/17
HBV DNA	7.39 (5.42, 8.48)	6.91 (6.08,7.89)
Fibrosis stage (0–1/2/3/4)	146/116/67/51	0/41/25/16
Liver necroinflammatory activity (0–1/2/3)	173/146/48	57/21/4

Abbreviation: PLT = platelet; TP = Total Protein; ALB = albumin; PT = prothrombin time; GP73 = Golgi protein 73;TBIL = total bilirubin;TBA=; total bile acids, TBA; # not available;

Table 2
Predictive variables for liver necroinflammation by spearman ananlysis and multivariate analysis in patients with chronic hepatitis B infection.*

Variables	Univariate analysis		Multivariate analysis
	rho	*p*-value	*p*-value
Age(years)	−0.085	0.103	–
PT	0.288	0.000	–
TP	0.049	0.356	–
Alb	−0.295	0.000	–
ALT(U/l)	0.469	0.000	–
AST(U/l)	0.551	0.000	0.000
GP73	0.524	0.000	0.000
GGT	0.618	0.000	0.000
AKP	0.237	0.000	0.009
TBA	0.422	0.000	–
TBIL	0.192	0.000	–
DBIL	0.284	0.000	–

* Abbreviation: TP = Total Protein; ALB = albumin; PT = prothrombin time; GGT = gamma glutamyl transpeptidase; AKP = alkaline phosphatase; TBA = total bile acids.

According to the results from multivariate analysis, a series of diagnostic models for moderate liver necroinflammation were constructed as followed. At first, all of these four independent factors (AST + GGT + GP73 + AKP) were included in the model and this model was defined as reference model (RM) as $\frac{1}{1+e^{-(-3.383+0.016\times GP73+0.026\times AST+0.036\times GGT-0.007\times AKP)}}$.

Next, we tried to remove single or multiple variables by binary logistic regression, to simplify the calculation process with mild or no reduction of diagnostic value. The simplified models were defined as follow: $\frac{1}{1+e^{-(-3.631+0.014\times GP73+0.023\times AST+0.034\times GGT)}}$ (AKP removed model, ARM), $\frac{1}{1+e^{-(-2.689+0.025\times AST+0.041\times GGT)}}$ (AKP and GP73 removed model, AGRM).

3.3. Comparison of diagnostic values between new models and single clinical parameter for identification of patients with moderate to severe liver necroinflammatory activity

The comparison of AUROC curves were used to evaluate the diagnostic performance for the improvement of new models. As showed in Table 3, three new models, RM, ARM and AGRM all exhibited significant better diagnostic value for moderate liver necroinflammation compared to using GP73, ALT or AST alone ($p < .001$). Among these three models, RM exhibited highest diagnostic value for moderate liver necroinflammation (AUROC = 0.898, 95%CI = 0.862–0.927), and ARM was the second best (AUROC = 0.890, 95%CI) with no statistical difference to RM (p = NS). Whereas, AGRM, which removed both AKP and GP73, displayed significant reduction of diagnostic performance compared to RM (p = .0407). Thus, we redefined ARM ($\frac{1}{1+e^{-(-3.631+0.014\times GP73+0.023\times AST+0.034\times GGT)}}$), which combined AST, GP73 and GGT, as a hepatic inflammation model (HIM) to reflect the presence of moderate and severe hepatic inflammation in CHB patients.

Considering that HBeAg status was regarded as an important indicator of the disease phrasing for CHB patients, subgroup analysis according to HBeAg status was also investigated. The clinical characteristics of patients with different HBeAg status were shown in Supplementary Table 1 and Supplementary Fig. 1. As showed in Table 4, HIM maintained excellent diagnostic performance both in HBeAg positive group (AUROC = 0.891, 95%CI = 0.846–0.926) and HBeAg negative (AUROC = 0.887, 95%CI = 0.809–0.941) group.

Meanwhile, the power of GP73 to detect patients with active necroinflammatory liver damage (HAI score > 4) was confirmed in the Friendship cohort patients with significant fibrosis (Ishak > 2). With the AUROC as high as 0.857 (95%CI: 0.717–0.894), HIM performed better than using ALT (AUROC: 0.817, 95%CI: 0.717–0.897), AST (AUROC: 0.826, 95%CI:0.724–0.902) or GP73 alone (AUROC: 0.831, 95%CI: 0.730–0.906) although no significant different was observed due to the sample numbers.

With the AUROC as 0.823 (95%CI: 0.721–0.900), HIM performed better than using ALT (AUROC: 0.778, 95%CI: 0.671–0.864), AST (AUROC: 0.753, 95%CI:0.643–0.843) or GP73 alone (AUROC: 0.815, 95%CI: 0.721–0.900) although no significant different was observed

Table 3
Diagnostic values of GP73, AST, ALT and new models for patients with moderate liver inflammatory activity.

Status	Variable	AUROC	95%CI	Cut-off value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)	*p*
Total										
	GP73	0.803	0.758–0.842	79.79	80.41	68.79	74.3	75.8	74.93	< 0.0001
	AKP	0.637	0.585–0.687	66	78.42	44.24	61.8	64.0	62.53	< 0.0001
	ALT	0.771	0.725–0.813	40	78.35	54.34	65.8	69.1	67.03	< 0.0001
	AST	0.819	0.775–0.857	45	70.62	81.29	81.1	70.9	75.62	< 0.0001
	RM(190/165)	0.898	0.862–0.927	0.3707	86.84	78.79	82.5	83.9		< 0.0001
	ARM	0.890	0.853–0.921	0.4284	81.05	81.93	83.7	79.1		< 0.0001
	AGRM(190/166	0.879	0.841–0.911	0.5186	74.74	87.35	86.1	75.1		< 0.0001

Abbreviation: GP73 = Golgi protein 73, ALT = alanine transaminase, AST = Aspartate transaminase, ARM = AKP removed model, AGRM = AKP and GP73 removed model.

Table 4
Diagnostic values of GP73, AST, ALT and HIM for patients with moderate liver inflammatory activity.

Status	Variable	AUROC	95%CI	Cut-off value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)	*p*
HBeAg										
Positive	GP73	0.782	0.728–0.831	97.99	66.89	80.53	82.1	64.5	72.73	< 0.0001
	ALT	0.760	0.704–0.810	40	82.78	44.25	66.5	65.8	66.31	< 0.0001
	AST	0.820	0.768–0.864	64	59.60	91.96	90.9	62.8	73.38	< 0.0001
	HIM(147/107)	0.891	0.846–0.926	0.4471	84.35	78.50	84.4	78.5		< 0.0001
Negative	GP73	0.841	0.755–0.905	75.25	83.72	76.67	72.0	86.8	79.61	< 0.0001
	ALT	0.767	0.673–0.845	40	62.79	73.33	62.8	73.3	68.93	< 0.0001
	AST	0.814	0.725–0.884	41	55.81	93.22	85.7	74.3	77.45	< 0.0001
	HIM(43/59)	0.887	0.809–0.941	0.3307	86.05	83.05	78.7	89.1		< 0.0001

Abbreviation: GP73 = Golgi protein 73, HIM = hepatic inflammation model.

due to the sample numbers.)

3.4. HIM could identify moderate to severe liver nercoinflammatory activity in CHB patients with ALT < 1 × ULN (40 U/l)

According to the diagnosis of liver biopsy, 78 (57.35%) of the 136 patients in this study were suffering either from moderate liver inflammatory activity or significant and/or worse fibrosis but with ALT ≤ 40 U/l. It is of interesting to explore the diagnostic value of HIM for patients with different ALT concentrations, particularly those with ALT less than the traditional upper concentration 40 U/l.

More promising, HIM maintained the excellent diagnostic performance in CHB with ALT < 40 U/l as well as for all patients (Table 5). The AUROC was as high as 0.873 (95%CI:0.804–0.924) which is much better than using AST (P = .0038) or GP73(P = .0535) alone. Further substrate statistical analysis showed that the diagnosis performances of HIM seemed won't be affected by HBeAg status of patients (Table 5).

3.5. Serum GP73 was the important source of diagnostic value improvement for HIM

According to result 3, removal of GP73 from model could significantly reduce diagnostic accuracy for liver necroinflammation model, which means GP73 was the important source of the diagnostic value improvement for HIM. Meanwhile, in ALT < 40 U/l patients, the AUROC of serum GP73 was 0.821 (95%CI: 0.746–0.880), with a 66.67% sensitivity and a 86.17% specificity in identifying the patients with moderate to severe liver necroinflammatory activity (Table 5). This result also indicated that serum GP73 was probably a reliable supplementary biomarker of ALT to evaluate liver lesions and to increase the diagnostic accuracy for HIM. Unlike ALT, AST and GGT, which are all the liver enzyme that could be released from hepatic parenchymal cells while the cells damage happening, GP73 was reported as the Golgi membrane that can be sliced by proprotein convertase furin to release into blood. In order to explored the relationship between serum GP73 and liver necroinflammation, a series statistical analyses were performed as follow.

Box plot was draw to further evaluate the correlation between serum GP73 and liver necroinflammatory lesion. As shown in Fig. 1A, the median serum GP73 concentration in chronic HBV infected patients [86.40 (61.79, 123.36) ng/ml] was significantly higher than that in healthy control [35.07 (24.97–45.37) ng/ml]. Further analysis showed that concentrations of serum GP73 increased with the worsening of liver inflammatory activity [A1, 66.67 (52.42, 87.27); A2, 105.29 (78.40, 140.10); A3, 129.53 (107.61, 178.62) ng/ml], in a stepwise manner (Fig. 1B). These results indicated that serum GP73 may have potential to reflect the progression of liver injury in those chronic HBV infected individuals.

To further confirm that the aberrantly increase of serum GP73 associated with the severity of liver inflammation, the dynamic changes of GP73 among a longitudinal cohort of 39 patients underwent Entecavir treatment for liver fibrosis/cirrhosis were observed [20]. As revealed by histological evaluation (Fig. 1C), in contrast to the slightly improved fibrosis scores (2.54 ± 0.13 vs 2.13 ± 0.14, p = .046), a significant decline of liver necroinflammatory activity from 1.56 ± 0.10 at baseline to 0.85 ± 0.06 (p < .001) was observed (Fig. 1C). Meanwhile, the concentrations of serum GP73 were also showed a dramatic decrease from 92.49 (59.04, 114.11) ng/ml to 42.20 (30.91, 54.72) ng/ml (p < .001), in a pattern similar to the dynamic change of ALT and AST activity (Fig. 1D). Taken together, the results here suggested that the decline of GP73 is probably consistent with the improvement of liver inflammation.

4. Discussion

Liver histological lesion is widely accepted as an important indication for chronic HBV infected individuals to receive anti-viral treatment. To identify these patients, non-invasive serum biomarkers with power to identify whether an individual suffering moderate liver injury is urgently needed, especially in those CHB patients with no increased ALT. Previously, some studies have also established diagnostic models to predict inflammation. However, most of them based on four or more

Table 5
Diagnostic values of GP73, AST and HIM for moderate liver inflammatory activity in ALT < 40 U/l patients.

ALT normal patients		AUROC	95%CI	Cut-off value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)	*p*-Value
Total	GP73	0.821	0.746–0.881	86.00	66.67	86.17	68.5	85.3	80.14	< 0.0001
	AST	0.754	0.672–0.824	23	83.33	55.91	46.1	88.1	64.44	< 0.0001
	HIM(42/91)	0.873	0.804–0.924	0.2600	78.57	84.62	70.2	89.5		< 0.0001
HBeAg										
Positive	GP73	0.828	0.725–0.905	86.00	73.08	86.00	73.1	86.0	81.58	< 0.0001
	AST	0.732	0.617–0.827	26	69.23	75.51	60.0	82.2	73.33	0.0002
	HIM(26/47)	0.883	0.786–0.946	0.2294	88.46	78.72	69.7	92.5		< 0.0001
Negative	GP73	0.804	0.681–0.895	86.00	56.25	86.36	60.0	84.4	78.33	< 0.0001
	AST	0.791	0.667–0.885	22	93.75	61.36	46.9	96.4	70.00	< 0.0001
	HIM(16/44)	0.854	0.739–0.932	0.2659	75.00	86.36	66.7	90.5		< 0.0001

Abbreviation: GP73 = Golgi protein 73, ALT = alanine transaminase, AST = Aspartate transaminase, HIM = hepatic inflammation model.

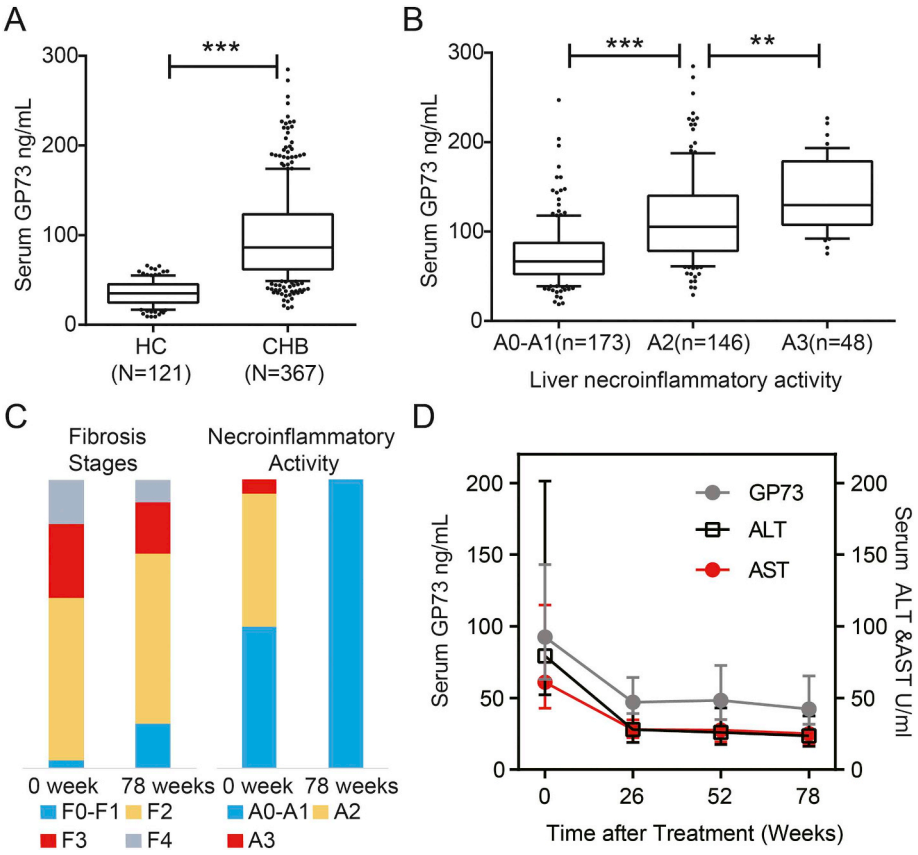

Fig. 1. The serum GP73 concentration associated with liver nercroinflammation and fibrosis. In 302 Military Hospital of China cohort (A) Serum GP73 increased in chronic hepatitis B patients. (B) Serum GP73 concentration in patients with different liver necroinflammatory activity. In longitudinal cohort (C) liver fibrosis stage, liver necroinflammatory scores, (D) serum GP73 (median ± IQR), AST and ALT concentration change during treatment with ETV. *$p < .05$, **$p < .01$, ***$p < .001$.

clinical index, which probably leads to the difficulty of calculation and increase of cost [27207016; 29914513]. And the model based on two indexes usually exhibit poor diagnostic value [30542389] [Besides, few of previous study has examined the stability of the diagnostic model both in general CHB patients and ALT < 40 U/l subgroup. Whereas, HIM in this study only based on AST, GGT and GP73 but exhibited stable and accurate diagnostic performance for moderate liver necroinflammation both in general CHB patients and ALT < 40 U/l subgroup.

In this retrospective cohort study, we demonstrated that, HIM, a serum GP73 based model, could act as an auxiliary biomarker for moderate liver necroinflammation, especially in ALT < 40 U/l individuals with chronic HBV infection. More than that, the study suggested that through HIM, 81.05% patients accompanied with ≥A2 could be confirmed without liver biopsy, with PPV as high as 83.7% and NPV as high as 79.1%. Meanwhile, the status of HBeAg was not the interference factor for the diagnostic value of HIM in CHB cohort.

In current study, serum GP73, as a major source of improvement for diagnostic model's accuracy, has been further explored in outpatients with chronic hepatitis B infection with different liver necroinflammation activity, and the relationship between GP73 and liver necroinflammation was confirmed from various aspects. First of all, the aberrant elevation of serum GP73 concentrations was observed in parallel with liver necroinflammation worsening, just as previously reported by others [6, 23]. Also, this elevation of serum GP73 with liver necroinflammation is fibrosis independent due to the fact that GP73 was increased in patients with liver necroinflammation alone. Second, a positive correlation between serum GP73 and liver inflammatory activity was supported by the fact that concentrations of serum GP73 were closely correlated with various parameters for liver injury, which was further supported by the observation of dramatically decrease of serum GP73 concentrations after the control of liver inflammation archived by antiviral treatment. In addition, with AUROC as high as 0.857, the power of GP73 to reflect moderate necroinflammatory liver damage (HAI score > 4) was confirmed in the Friendship cohort patients with significant fibrosis (Ishak > 2). All these data suggested that serum GP73 can be a candidate to indicate liver necroinflammation.

We must acknowledge that the use of HIM as an auxiliary biomarker for the progression of liver disease still needs verification in large queue.

In conclusion, HIM could act as an additional non-invasive biomarker to identify at least moderate liver necroinflammation. The application of HIM in CHB patients with ALT < 40 U/l could help us to identify CHB patients with moderate liver necroinflammation which is vital for initiation of timely anti-viral treatment and preventing the progression of chronic hepatitis B.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cca.2019.02.019.

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