Plasma Golgi protein 73 levels predict prognosis of HCV-related hepatic fibrosis

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Summary. Objectives. To explore the correlation between plasma Golgi protein 73 (GP73) and progression of hepatitis C virus (HCV)-induced hepatic fibrosis.

Methods. A total of 232 subjects of chronic hepatitis C and 31 healthy controls were enrolled from the Third Hospital of Hebei Medical University from January 2010 to September 2018. The plasma GP73 levels were detected by ELISA. Liver tissue sections stained with hematoxylin and eosin and Masson-trichrome were examined under a light microscope based on the METAVIR scoring system and "Beijing classification (P-I-R)". The correlation analysis and receiver operating characteristic (ROC) curve were performed to analyze the diagnostic efficiency of plasma GP73, APRI, and FIB-4 for staging hepatic fibrosis and predicting the disease progression.

Results. The plasma GP73 levels were increased with the progression of liver fibrosis, and GP73 concentrations in healthy controls, HCV patients with fibrosis stage 1, 2, 3 and 4 were 94.82, 151.3, 157.9, 181.7 and 208.5 ng/ml, respectively. According to "Beijing classification", There was a statistically significant difference in plasma GP73 concentrations between patients in the progression and regressive / indeterminate group (177.08 vs 154.00 ng/ml , P = 0.007).The area under the ROC curves (AUCs) of GP73, APRI, and FIB-4 for diagnosis of liver cirrhosis were 0.89, 0.77, and 0.82, respectively, and GP73 for progressive fibrosis was 0.73. The plasma GP73 levels were significantly positively correlated with hepatic inflammation, serum ALT, and negatively correlated with albumin levels.

Conclusion. The plasma GP73 might be a potential biomarker for staging liver fibrosis, and could predict regression or progression of HCV-related liver fibrosis.

Key words: Hepatitis C, Golgi protein 73, APRI; FIB-4, Liver fibrosis, Hepatic inflammation, Prognosis

Introduction

In the era of direct-acting antiviral drugs (DAAs) agents, the elimination of hepatitis C viral (HCV) infection is achievable. However, 23% of patients with chronic HCV infection develop advanced liver fibrosis or cirrhosis, which is a hard nut by the short-term antiviral therapy (Wei, 2018; Taherkhani and Farshadpour, 2017). To crack this nut in these patients, an accurate diagnosis is essential and helpful. Early

Abbreviations. GP73, Golgi protein 73; HCV, hepatitis C virus; CHC, chronic hepatitis C; APRI, aspartate aminotransferase to platelet ratio index; FIB-4, fibrosis index based on four factors; ELISA, enzyme-linked immunosorbent assay; ROC, receiver operating characteristic; HIV, human immunodeficiency virus; qRT-PCR, reverse transcription-quantitative polymerase chain reaction; ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma glutamyl transpeptidase; PLT, platelet; H & E, hematoxylin and eosin; LSM, liver stiffness measurements; BMI, body mass index; AUC, areas under the receiver operating characteristic curve; CLC, compensated liver cirrhosis; DLC, decompensated liver cirrhosis.

diagnosis and effective treatment are beneficial to inhibit or even reverse the progression of HCV-related hepatic fibrosis. The "Beijing classification" for liver fibrosis proposed by Sun et al provides a crucial basis for the prognosis of fibrotic chronic hepatitis C (CHC) (Sun et al., 2017). Based on it, the establishment of a novel, rapid and non-invasive diagnostic method would increase the accuracy of early diagnosis, treatment, and prognosis of HCV-induced liver fibrosis.

Recent studies reported that Golgi protein 73 (GP73) is significantly associated with the development of liver fibrosis (Mao et al., 2010; Yao et al., 2018; Lu and Zhang, 2018). We performed a case-control study to clarify the relationship between plasma GP73 levels, the progression, and prognosis of HCV-related hepatic fibrosis according to the METAVIR scoring system and the "Beijing classification". Furthermore, the diagnostic efficiency of plasma GP73 in liver fibrosis was analyzed by the receiver operating characteristic (ROC) curve and compared with the clinical routine parameters: aspartate aminotransferase-to-platelet ratio index (APRI) (Wai et al., 2003) and the fibrosis index based on four factors (FIB-4) (Sterling et al., 2006).

Materials and methods

Subjects

A total of 232 patients with chronic HCV infection were recruited from the Third Hospital of Hebei Medical University from January 2010 to September 2018 (Fig. 1). Diagnoses were based on the Hepatitis C Guidance

2018 Update: AASLD-IDSA Recommendations for Testing, Managing and Treating Hepatitis C Virus Infection (AASLD-IDSA HCV Guidance Panel., 2018), and the Guideline of Prevention and Treatment for Hepatitis C by the Chinese Society of Hepatology and Chinese Society of Infectious Diseases and Parasitology of the Chinese Medical Association (Chinese Society of Hepatology and Chinese Society of Infectious Disease of Chinese Medical Association, 2015). Subjects with the following conditions were excluded: acute hepatitis C; co-infection with human immunodeficiency virus (HIV); co-infection with hepatitis A, B, or D virus. Thirty-one healthy controls were recruited from the physical examination center in the hospital. Written informed consents were obtained from all patients. The study was approved by the Ethics Committee of the Third Hospital of Hebei Medical University, according to the Declaration of Helsinki and Good Clinical Practice guidelines. All of the clinical data and blood samples of the HCV patients were collected at baseline, before they received antivirus therapies.

Detection of HCV antibodies and viral loads of HCV

Serum HCV antibody was detected by enzyme-linked immunosorbent assay (ELISA) using a commercial detection kit (Livzon Diagnostics Inc., Zhuhai, China). Plasma HCV RNA loads were determined by reverse transcription-quantitative polymerase chain reaction (qRT-PCR) assay which limit of detection is 15 IU/ml (Cobas Taqman HCV Test, Roche Diagnostics, Indianapolis, IN, USA).
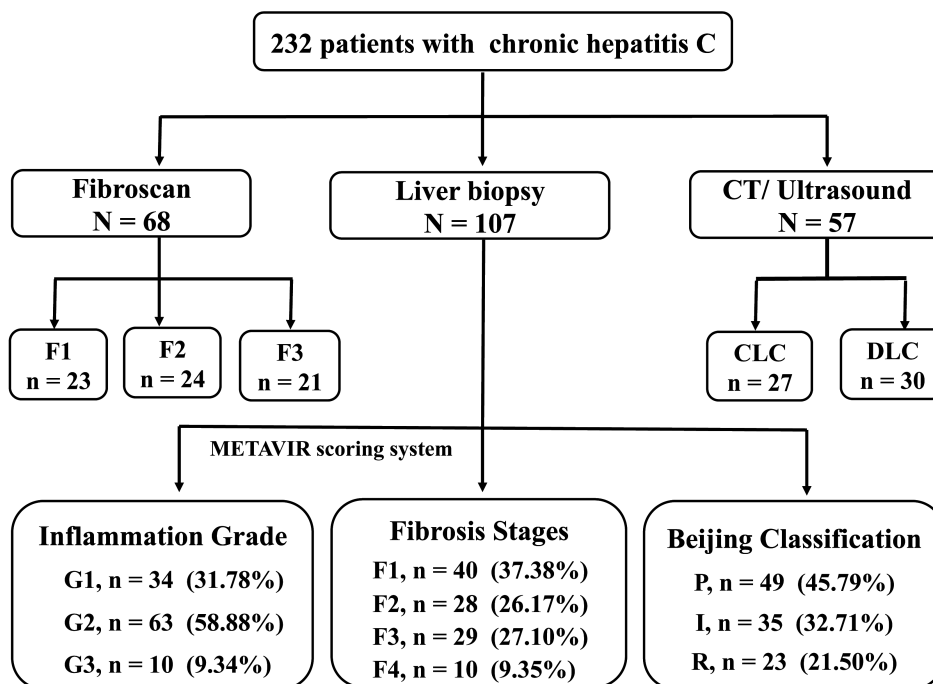

Fig. 1. The composition of enrolled patients. CLC, compensated liver cirrhosis; DLC, decompensated liver cirrhosis.

Biochemical assays

Serum albumin (ALB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma glutamyl transpeptidase (GGT) levels were tested using an Olympus AU5400 automatic chemical analyzer (Olympus CO., Ltd., Tokyo, Japan). Peripheral blood platelet (PLT) counts were detected with an automated hematology analyzer using the Hydro Dynamic Focusing method (XS 1000i; Sysmex Corporation, Kobe, Japan).

Test of plasma GP73 levels

Plasma GP73 levels were tested with ELISA according to the manufacturer's protocol (Hotgen Biotechnology Co., Ltd. Beijing, P.R. China).

Evaluation of liver histopathology

The ultrasound-guided percutaneous liver biopsy was routinely performed using a 16G liver puncture needle (Bard Peripheral Vascular, Inc., Tempe, AZ, USA). The liver biopsy specimens were considered reliable when the liver specimen length was ≥1.5 cm, or the portal tract number was ≥6. Liver tissue specimens were fixed in buffered formalin, embedded in paraffin, and then cut at a width of approximately 5µm and conventionally stained with hematoxylin and eosin (H&E) and Masson-trichrome. The liver sections were observed at 40x to 400x magnification using a Leica DM 2000 microscope (Leica Microsystems, Inc., Buffalo Grove, IL, USA). All of the liver samples were evaluated independently by two pathologists according to the METAVIR scoring system and the "Beijing classification", respectively.

Liver fibrosis staging

The stages of the liver fibrosis were classified according to the METAVIR scoring system (The French METAVIR Cooperative Study Group, 1994) as follows: F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis with rare septa; F3, many septa without cirrhosis; and F4, cirrhosis. Significant liver fibrosis was defined as ≥F2, and advanced fibrosis was ≥F3.

Quality of liver fibrosis

According to the "Beijing classification" (Sun et al., 2017), hepatic fibrosis is scored into three categories (P-I-R scores). Predominantly progressive (P) presents that more than 50% fibro-septal are wide/broad, loosely aggregated collagen fibers, which are moderate to markedly cellular containing, variably, inflammatory cells, macrophages, and ductular reactions. Predominately regressive (R) shows that more than 50% fibro-septal are thin and densely compacted stroma, which are largely acellular. Indeterminate (I) is defined as an uncertain mix/balance between progressive and

regressive scarring. If the examining pathologist cannot conclude as to whether progressive or regressive scarring predominates, or is tempted to reach higher magnification in order to assess the balance, the specimen is categorized as indeterminate (Fig. 2).

Liver stiffness measurements (LSM)

All subjects underwent LSM using FibroScan (EchoSens, Paris, France) on the right lobe of the liver, as previously described (Boursier et al., 2013). The results were expressed in kilopascals (kPa), and the median value of 10 acquisitions was used for analysis, including only cases with a successrate >60% and an interquartile range/median ratio <0.3. The same professionally trained physician performed all of these procedures.

APRI and FIB-4 scores

The aspartate aminotransferase to platelet ratio index (APRI) and the international normalized ratio index (FIB-4) were calculated according to the following formulas: APRI= [AST (U/L) / ULN (U/L)] ×100 / PLT (109/L), FIB-4 = Age (years) × AST (U/L) / PLT (109/L) × [ALT (U/L)]1/2, (ULN, upper limit of normal; the ULN of AST was 50 U/L for males and 40 U/L for females).

Statistical analysis

Statistical analysis was performed using SPSS 21.0 statistical software and Graphpad Prism 5.0. Normally distributed data were expressed as mean ± standard deviation (SD), skewed data as the median (interquartile range). Student's t-test or Mann-Whitney U test were used to compare the statistical differences between two groups. One-way analysis of variance (ANOVA) or Kruskal-Wallis H test was performed for comparisons of values among at least three groups; the least significant difference-t (LSD-t) test or Nemenyi method was conducted for further comparisons between any two groups. Correlations between variables were calculated using Spearman's rank order, and the receiver operating characteristic (ROC) curve with a 95% confidence interval (CI) was conducted by using Medcalc (15.6.1) to evaluate the diagnostic value. All P-values were two-tailed and considered significant when less than 0.05.

Results

Baseline characteristics of study subjects

The demographic, clinical characteristics, and plasma GP73 concentrations of the 232 patients with chronic hepatitis C and 31 healthy controls are shown in Table 1. As fibrosis stages progress, the age, the plasma GP73 and the liver function-associated biochemical indices, were significantly increased, while the serum

ALB levels and the peripheral blood PLT counts were decreased (P<0.05). The AFP levels were gradually increased with the progression of liver fibrosis, but lower than the upper limit of normal (≤7 ng/ml). There were no significant differences in gender or body mass index (BMI). Plasma GP73 levels in hepatitis C patients were significantly higher than those in the healthy controls (172.90 vs 94.82 ng/ml, P<0.001).

In addition, the demographic and clinical data of 68 patients who received LSM test are shown in table 2. Both the serum ALT and total bilirubin levels of the patients were within the upper limit of normal and did not significantly impact the LSM vaules, although our previous study demonstrated that elevated ALT and bilirubin (≥2 ULN) influenced LSM values (Wang et al., 2015) .

Relationship between plasma GP73 levels and liver fibrosis degrees

Plasma GP73 levels in chronic hepatitis C patients with different liver fibrosis stages
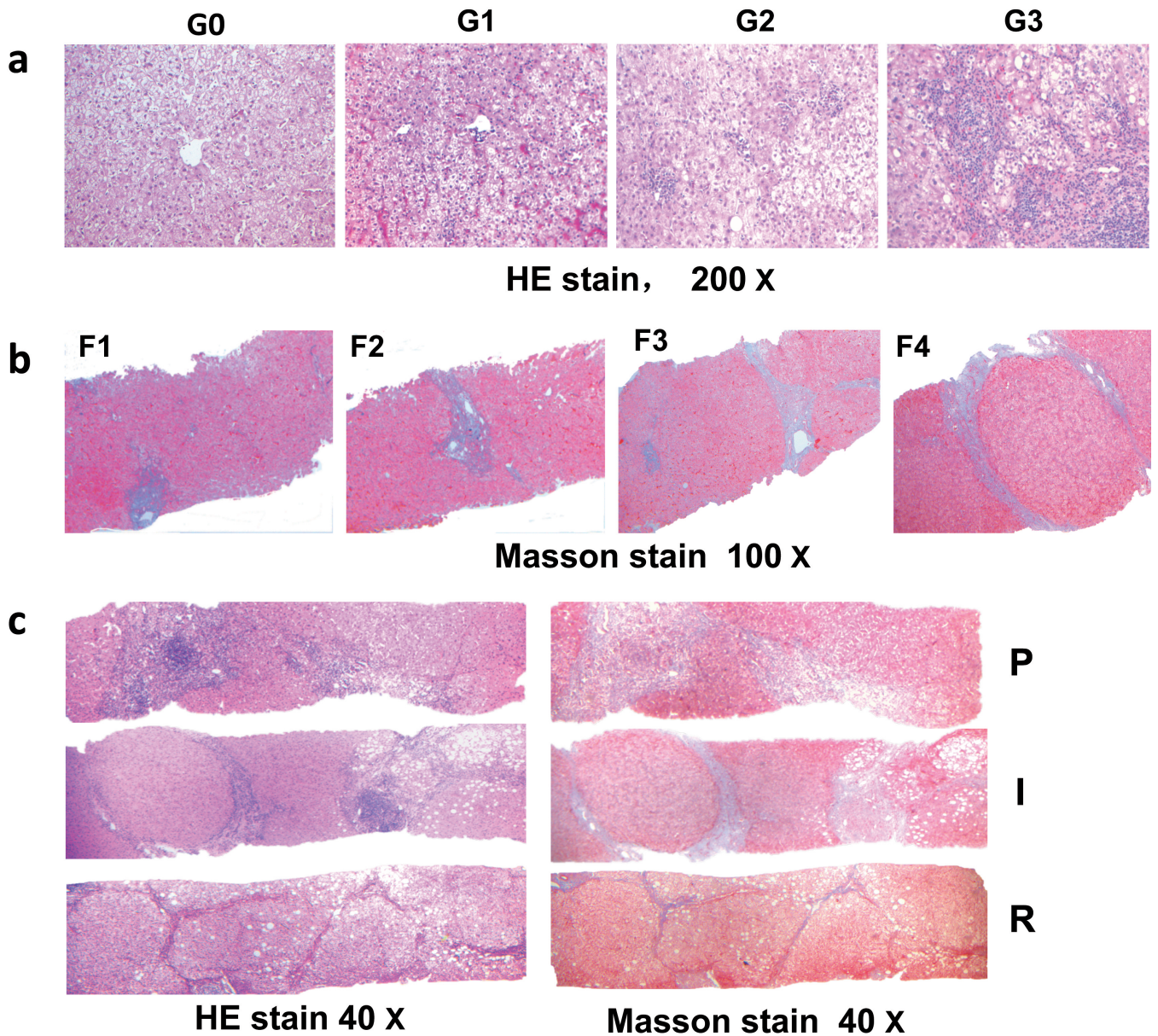

Fig. 2. Hepatic inflammation activity grades, fibrosis stages and P-I-R classification in the chronic hepatitis C patients. **a-b.** The different liver fibrosis stages assessed by H&E and Masson trichrome staining. **c.** P-I-R scores assessed by H&E and Masson. H&E: hematoxylin and eosin. a, x 200; b, x 100; c, x 40.

There was a significant difference in the plasma GP73 levels between the patients with HCV-related liver fibrosis and the healthy controls(P<0.001). Significant up-regulations of plasma GP73 levels were observed when the hepatic fibrosis stages (F1~4) increased (P<0.001). The plasma GP73 levels in F3 patients were markedly higher than those in F2 patients (P=0.003) (Table 1, Fig.3a).

To clarify the value of plasma GP73 as a diagnostic biomarker for liver fibrosis in patients with chronic hepatitis C, we compared the efficacy of the plasma GP73, APRI, and FIB-4 via the ROC curve. The areas under the ROC curves (AUCs) of GP73, APRI and FIB-4 were 0.73, 0.77, 0.81 for significant fibrosis (F2), 0.77, 0.75, 0.82 for advanced fibrosis (F3), and 0.89, 0.77, 0.82 for liver cirrhosis (F4), respectively (P<0.001) (Fig.

3b-d, Table 3).

Association between plasma GP73 levels and P-I-R categories

According to P-I-R categories, the patients with predominantly progressive hepatic fibrosis exhibited significantly higher levels of plasma GP73 than the patients with predominantly regressive and indeterminate hepatic fibrosis [177.08 (149.35~205.58) vs 154.00 (121.95~178.05) ng/ml, P = 0.004] (Fig. 3e). With a cut-off value set at 177.8 ng/ml in progressive fibrosis group (P), the AUC for plasma GP73 was 0.73 (95% CI: 0.64-0.81) with a sensitivity of 51.02% and a specificity of 86.21%, while APRI and FIB-4 had no predictive significance (Fig. 3f, Table 4).

Table 1. Clinical characteristics and plasma GP73 levels of the HCV patients and health controls.

Parameter	Healthy Control	Stages of hepatic fibrosis				F/X2	P
	n=31	F1 (n=63)	F2 (n=50)	F3 (n=50)	F4 (n=69)		
Gender (M/F)	15/16	30/33	24/26	25/25	22/47	4.78	0.189
Age, median (IQR)	47.50 (45.7, 56.00)	48.00 (32.00, 55.25)	51.00 (47.50, 56.50)	55.00 (49.00, 61.00)	59.00 (52.00, 65.50)	45.19	0.000
BMI (kg/m^2), median (IQR)	22.38 (20.69, 24.29)	24.03 (22.04, 26.37)	25.00 (23.19, 27.68)	25.56 (23.18, 27.34)	25.10 (23.18, 27.04)	3.39	0.335
ALB (mean ± SD, g/L)	44.92±2.61	46.36±4.29	44.13±4.12	43.77±4.21	38.53±6.12	23.31	0.000
ALT (U/L), median (IQR)	12.50 (10.00, 16.00)	22.00 (13.00, 41.00)	41.00 (19.50, 71.50)	32.50 (18.25, 67.00)	30.00 (18.00, 48.25)	8.12	0.044
AST (U/L), median (IQR)	16.00 (14.00, 19.00)	22.00 (18.50, 28.50)	41.00 (20.50, 60.00)	29.50 (22.00, 49.25)	36.50 (22.00, 52.75)	15.62	0.001
GGT (U/L), median (IQR)	18.00 (14.00, 20.25)	23.00 (16.00, 34. 00)	32.00 (19.50, 52.00)	40.00 (20.75, 68.00)	42.00 (24.00, 63.00)	8.30	0.040
PLT (mean ± SD,109/L)	220.59±49.08	192.50±57.83	163.56±43.96	156.39±47.37	102.00±57.30	33.97	0.000
AFP (mean ± SD, ng/ml)	-	2.90±1.11	3.25±1.51	3.21±1.68	3.58±1.51	4.094	0.009
GP73 (ng/ml) , median (IQR)	94.82 (85.39, 104.76)	151.30 (123.10, 170.10)	157.90 (132.00, 172.60)	181.70 (149.43, 219.05)	208.50 (164.44, 324.73)	54.70	0.000
APRI, median (IQR)	0.19 (0.12, 0.26)	0.30 (0.23, 0.47)	0.64 (0.29, 1.12)	0.49 (0.31, 0.81)	0.96 (0.61, 2.12)	61.99	0.000
FIB-4, median (IQR)	0.96 (0.79, 1.65)	1.01 (0.77, 1.73)	1.95 (1.01, 2.72)	1.95 (0.91, 2.37)	4.06 (2.48, 6.87)	75.12	0.000

Continuous variables were expressed as mean ± standard deviation (SD) or median and interquartile range (IQR). BMI, body mass index; TBIL, total bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma glutamyltransferase; ALB, albumin; PLT, platelet; AFP, α-fetoprotein; GP73, Golgi protein 73; APRI, The aspartate aminotransferase to platelet ratio index; FIB-4, the international normalized ratio index.

Table 2. Demographic and clinical characteristics of the patients who received liver stiffness testing.

Parameters	Stages of hepatic fibrosis			F/X2	P
	F1 (n=23)	F2 (n=24)	F3 (n=21)		
Gender (M/F)	12/11	12/12	9/12		
Age, median (IQR)	50 (31, 59)	54 (50, 56)	57 (52, 63)	1.327	0.025
BMI (kg/m^2), median (IQR)	24.04 (21.82, 25.93)	27.18 (24.03,29.07)	26.83 (22.73, 27.96)	0.400	0.527
ALB (mean ± SD, g/L)	46.57±3.37	45.96±3.40	42.91±5.30	3.102	0.057
ALT (U/L), median (IQR)	19.0 (10.5, 27.5)	24.0 (14.0, 38.5)	27.5 (20.5, 76.8)	4.847	0.089
AST (U/L), median (IQR)	21.0 (16.0, 34.5)	29.0 (15.5, 38.0)	37.0 (22.5,50.3)	4.649	0.098
GGT (U/L), median (IQR)	20.0(14.6, 35.0)	18.5 (15, 23.3)	28.5 (20.5, 71.5)	3.375	0.185
TB (μmol/L), median (IQR)	17.44 (11.49, 21.63)	14.55 (11.3, 20.55)	12.39 (10.20, 18.23)	2.854	0.240
PLT (mean ± SD,109/L)	178.09±48.92	151.00±37.04	142.00±39.92	1.481	0.238
GP73 (mean ± SD, ng/ml)	137.23±33.72	147.44±54.96	207.69±56.43	12.944	0.000
APRI, median (IQR)	0.29 (0.24, 0.55)	0.60 (0.27, 0.78)	0.63 (0.39, 0.81)	3.155	0.206
FIB-4, median (IQR)	1.44 (0.81, 2.46)	1.95 (1.25, 2.74)	2.09 (1.73,2.81)	0.834	0.659

Continuous variables were expressed as mean ± standard deviation (SD) or median and interquartile range (IQR). BMI, body mass index; TBIL, total bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma glutamyl transferase; ALB, albumin; PLT, platelet; GP73, Golgi protein 73; APRI, The aspartate aminotransferase to platelet ratio index; FIB-4, the international normalized ratio index.

Table 3. GP73, APRI and FIB-4 diagnosis efficacy in HCV fibrosis (ROC curve analysis).

Fibrosis stages/Items		Cut-off	AUC	Se, %	Sp, %	95%CI	P
F≥2 (n=169)	GP73	179.00	0.73	51.48	85.71	0.668,0.786	0.001
	APRI	0.55	0.77	65.71	83.67	0.707,0.831	0.001
	FIB-4	1.84	0.81	74.44	80.85	0.746,0.865	0.001
F≥3 (n=119)	GP73	179	0.77	61.03	81.42	0.712,0.824	0.001
	APRI	0.73	0.75	56.86	82.61	0.687,0.812	0.001
	FIB-4	1.66	0.82	84.00	65.96	0.756,0.870	0.001
F≥4 (n=69)	GP73	206.3	0.89	81.40	84.05	0.836,0.927	0.001
	APRI	0.84	0.77	61.90	82.11	0.702,0.835	0.001
	FIB-4	2.14	0.82	83.33	67.77	0.753,0.876	0.001

GP73, Golgi protein 73; APRI, The aspartate aminotransferase to platelet ratio index; FIB-4, the international normalized ratio index. AUC, areas under the receiver operating characteristic curve.

Table 4. GP73, APRI and FIB-4 in diagnosis of the progressive fibrosis (ROC curve analysis).

values	Cut-off	AUC	95%CI	Se, %	Sp, %	P
GP73 (ng/ml)	177.80	0.73	0.636,0.811	51.02	86.21	0.0001
APRI	0.66	0.60	0.479,0.713	32.76	93.33	0.1888
FIB-4	1.20	0.59	0.463,0.712	63.46	61.54	0.3257

GP73, Golgi protein 73; APRI, The aspartate aminotransferase to platelet ratio index; FIB-4, the international normalized ratio index. AUC, areas under the receiver operating characteristic curve.
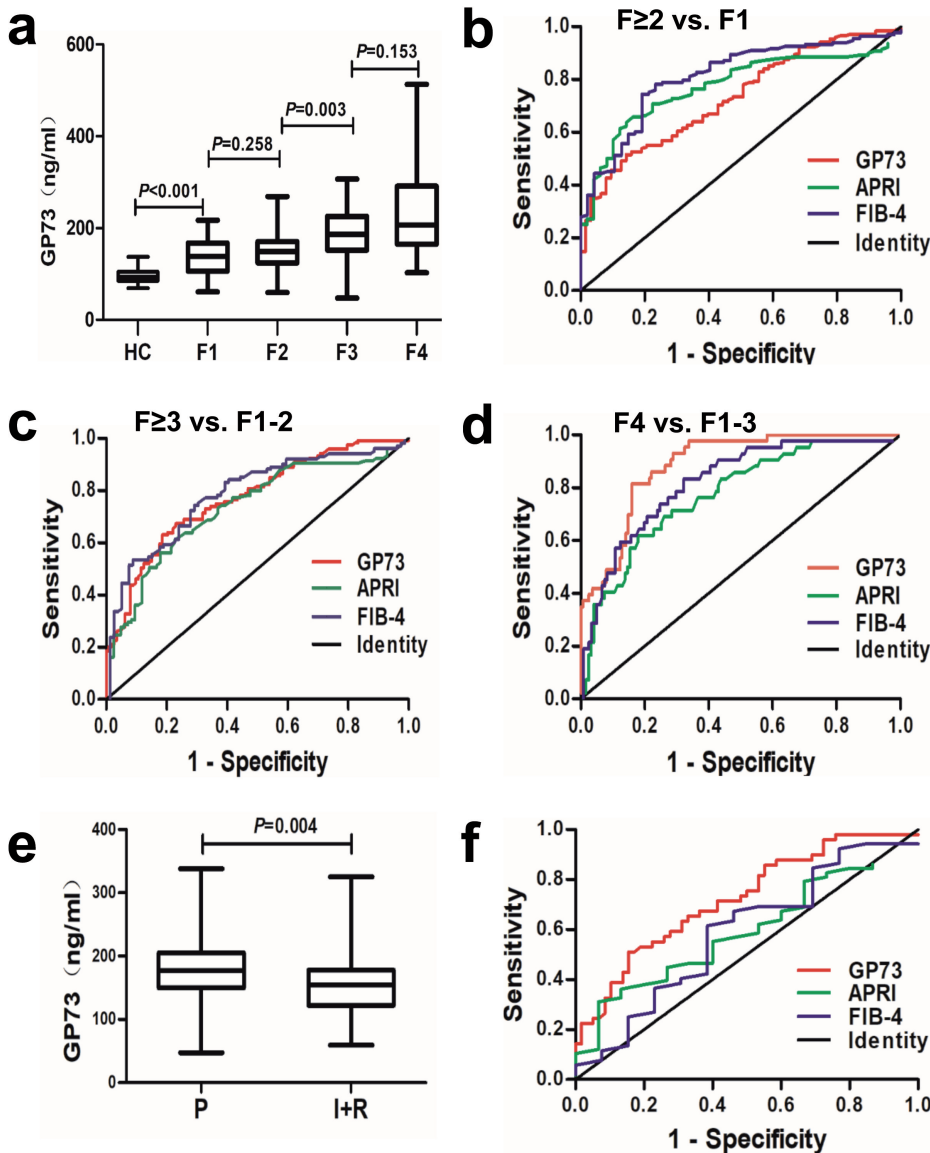
Fig 3. Association of plasma GP73 levels and stages of liver fibrosis. **a.** Differences of plasma GP73 levels among the health controls (HC) and HCV patients with different fibrosis stages, which were significant different between HC and HCV patients, and with different degrees of hepatic fibrosis. **b-d.** The analysis of ROC curves indicated that plasma GP73 AUCs were gradually increased with progression of liver fibrosis stages. **e.** The comparison of plasma GP73 between the P and I/R groups. **f.** ROC curves of plasma GP73, FIB-4 and APRI for diagnosis of progressive fibrosis.

Correlations of plasma GP73 with hepatic function reservation

Plasma GP73 levels in patients with HCV-related compensated liver cirrhosis (CLC) were significantly lower than those in patients with decompensated liver cirrhosis (DLC)[189.16 (163.65~221.70) vs 232.50 (174.60~335.65) ng/ml, P=0.023] (Fig. 4a). In terms of the serum ALB levels, the plasma GP73 levels were significantly higher in the patients with ALB<35g/L than those in patients with ALB≥35g/L (264.30vs 165.46 ng/ml, P<0.001, Fig. 4b). The results of the correlation analysis indicated that plasma GP73 levels were negatively correlated with PLT counts (r=-0.497) and serum ALB levels (r=-0.476, Fig. 4c,d).

Correlation of plasma GP73 levels with hepatic inflammation
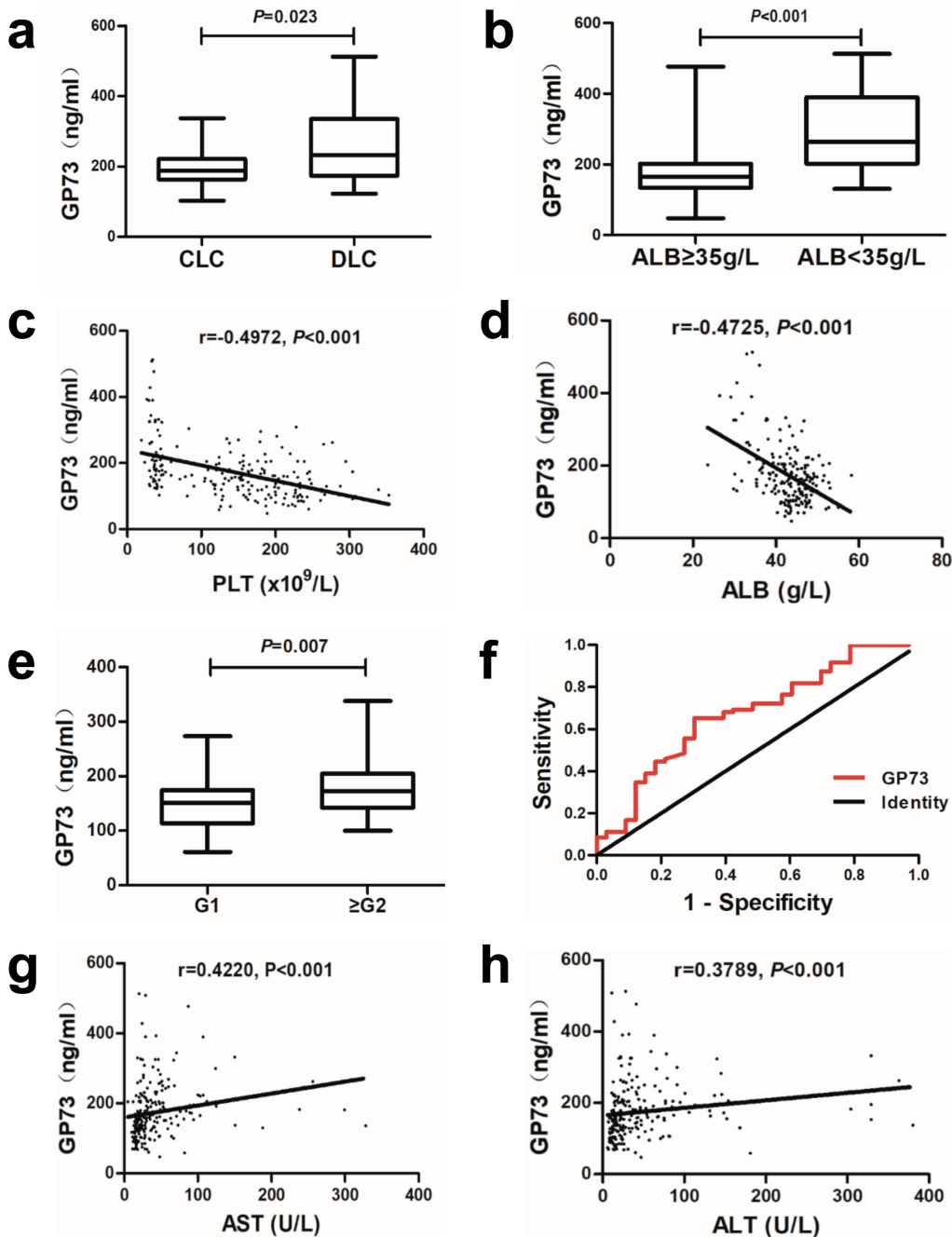
With the progression of hepatic inflammation, the

Fig. 4. Relationship of plasma GP73 levels and liver synthesis function and inflammation. The plasma GP73 levels were correlated with liver function reservation **(a, b)** and inflammation grades **(e)**, negatively correlated with serum ALB levels and peripheral blood PLT counts **(c, d)** and positively correlated with ALT and AST **(g, h)**. **f.** ROC curve of plasma GP73 for diagnosing inflammation grade ≥2.

plasma GP73 levels were increased gradually, the mean values were 157.55 (127.80~178.69) ng/ml in inflammatory degree G1 and 172.15 (142.65~204.85) ng/ml in ≥G2 groups, respectively (P=0.007, Fig. 4e). ROC analysis in ≥G2 patients showed that the AUC was 0.68 (95% CI: 0.54-0.73). The sensitivity (Se) was 65.28%, the specificity (Sp) was 69.70%, and the cut-off value was 158.4 ng/ml (Fig. 4f). Through correlation analysis, plasma GP73 levels were positively correlated with serum ALT (r=0.422) and AST levels respectively (r=0.364, Fig. 4g,h).

Discussion

GP73, a resident Golgi-specific transmembrane glycoprotein, is normally expressed in biliary epithelial cells (Mao et al., 2010) and anchored to the cis-Golgi body membrane through a membrane-producing domain. The hydrolysis reaction occurs under the action of furin, and the ectodomain of GP73 is cleaved and released into the extracellular fluid to form serum GP73. It is reported that GP73 is an effective and reliable serological marker for the diagnosis of advanced fibrosis and prediction of the appearance of cirrhosis in patients with chronic hepatitis B (Cao et al., 2017; Liu et al., 2018; Yao et al., 2018). GP73 levels were elevated in the activated hepatic stellate cells (Iftikhar et al., 2004). Our study showed that the plasma GP73 levels in hepatitis C patients were significantly higher than those in the healthy controls, and gradually increased as stage-constant fibrosis progress. The ROC curve analysis presented that the diagnostic efficiency of GP73 for liver cirrhosis was 0.89, with sensitivity 81.40% and specificity 84.05%. One study performed by Qian et al. reported the lower efficiency of GP73 diagnosis HCV-related liver cirrhosis, in which the value is limited compared with APRI and FIB-4 (Qian et al., 2019). Further, there were significant differences in plasma GP73 levels between patients with compensated liver cirrhosis and those with decompensated liver cirrhosis. It demonstrated that plasma GP73 levels were associated with chronic HCV infection and the development of HCV-related liver fibrosis.

In our study, liver sections were classified into three types according to the "Beijing classification, P-I-R categories" of liver fibrosis to investigate whether plasma GP73 could predict the progression or regression of HCV-related liver fibrosis (Sun et al., 2017). The results presented that the plasma GP73 levels in patients with predominantly progressive (P) hepatic fibrosis were significantly higher than those in patients with predominately regressive (R) / indeterminate (I) hepatic fibrosis, and the levels of plasma GP73 were elevated in parallel with the severity of hepatic fibrosis from stage 1 to stage 4, especially in liver cirrhosis. Our findings suggested that GP73 could serve as a novel biomarker to predict regression or progression of HCV-induced hepatic fibrosis, as in HBV-induced hepatic fibrosis (Wei et al., 2013; Xu et al., 2015a,b), and might be an

essential tool in the therapy management of liver disease. Mauro et al. (2018) found that the presence of thick fibrous septa in patients may indicate the progression of fibrosis. D'Ambrosio and colleagues (D'Ambrosio et al., 2018) reported that the incidence of hepatocellular carcinoma in progressive fibrosis patients was significantly higher compared with that in regression or stable fibrosis patients after achieving sustained virological response (SVR). The APRI and FIB-4 scoring systems appeared to have no predictive prognostic value over the P-I-R categories of hepatic fibrosis, even though they were recommended in the diagnosis of advanced liver fibrosis. Plasma GP73 concentrations contribute to the recognition and benefit the physician in managing progressive hepatitis C patients.

Apart from the degree of liver fibrosis, we also explored the correlation between the plasma GP73 and liver function reservation. The GP73 levels in patients with ALB<35g/L were much higher than those in patients with ALB≥35g/L. Spearman's correlation analysis showed that plasma GP73 levels were negatively correlated with ALB and the peripheral blood PLT counts. These changes were consistent with decompensated liver cirrhosis. It further demonstrated that plasma GP73 levels were increased with the progression of liver injury, especially liver cirrhosis, and the deterioration of liver function reservation (Liu et al., 2018; Qian et al., 2019), thus, based on our results, high plasma GP73 levels (more than 264.3 ng/ml) may potentially indicate a poor prognosis for HCV-related liver cirrhosis.

Our previous studies (Wang et al., 2015, 2016) had found that hepatic inflammation, elevated serum ALT, AST, and bilirubin influence diagnosis values of APRI and FIB-4. Some other studies preferred GP73 to be a new effective biomarker for diagnosing liver necro-inflammation (Xia et al., 2019; Wei et al., 2019; Xu et al., 2015a,b, 2018). Does the hepatic inflammation also influence hepatic GP73 expression and increase the levels of peripheral blood GP73 concentration? Based on histopathology of the livers, this study showed that plasma GP73 levels were up-regulated in patients with remarkable hepatic inflammation in chronic hepatitis C patients. A similar tendency of GP73 appeared in patients with elevated ALT. It is reported that along with reduced liver necroinflammation, the serum GP73 concentration also gradually declined in chronic hepatitis B patients (Xu et al., 2015a,b). Therefore, when GP73 was used to diagnose liver fibrosis, the degree of hepatic inflammation should be considered, and repeated tests of GP73 are necessary when the patient has recovered from necroinflammatory liver injury.

In addition, there are still limitations of our study, such as unmeasured potential biases due to this retrospective cohort from only one center. Meanwhile, the application of P-I-R classification in the assessment of liver fibrosis is also inadequate. Thus, multicentered prospective studies with a larger cohort are necessary in

the future to evaluate the diagnostic potency of GP73.

In conclusion, plasma GP73 might be an important biomarker for diagnosis, evaluation of regression or progression of liver fibrosis, as well as an independent prognostic predictor of chronic hepatitis C and hepatic decompensation of patients with liver cirrhosis. A large scale of clinical studies on the application of the plasma GP73 alone and combined with APRI, FIB-4, or liver stiffness measurement would be of benefit to the diagnosis and therapy of liver fibrosis and the early stages of cirrhosis.

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