

Original Article

Correlation of serum liver fibrosis markers with severity of liver dysfunction in liver cirrhosis: a retrospective cross-sectional study

Cuihong Zhu*, Xingshun Qi*, Hongyu Li, Ying Peng, Junna Dai, Jiang Chen, Chunlian Xia, Yue Hou, Wenwen Zhang, Xiaozhong Guo

Liver Cirrhosis Study Group, Department of Gastroenterology, General Hospital of Shenyang Military Area, 83 Wenhua Road, Shenyang 110840, China. *Equal contributors.

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Abstract: Hyaluronic acid (HA), laminin (LN), amino-terminal pro-peptide of type III pro-collagen (PIIINP), and collagen IV (CIV) are four major serum markers of liver fibrosis. This retrospective cross-sectional study aimed to evaluate the correlations of the four serum markers with the severity of liver dysfunction in cirrhotic patients. Between January 2013 and June 2014, a total of 228 patients with a clinical diagnosis with liver cirrhosis and without malignancy underwent the tests of HA, LN, PIIINP, and CIV levels. Laboratory data were collected. Child-Pugh and model for the end-stage of liver diseases (MELD) scores were calculated. Of them, 32%, 40%, and 18% had Child-Pugh class A, B, and C, respectively. MELD score was 7.58 ± 0.50 . HA (coefficient r: 0.1612, $P=0.0203$), LN (coefficient r: 0.2445, $P=0.0004$), and CIV (coefficient r: 0.2361, $P=0.0006$) levels significantly correlated with Child-Pugh score, but not PIIINP level. Additionally, LN (coefficient r: 0.2588, $P=0.0002$) and CIV (coefficient r: 0.1795, $P=0.0108$) levels significantly correlated with MELD score, but not HA or PIIINP level. In conclusions, HA, LN, and CIV levels might be positively associated with the severity of liver dysfunction in cirrhotic patients. However, given a relatively weak correlation between them, our findings should be cautiously interpreted and further validated.

Keywords: Liver, fibrosis, cirrhosis, Child-Pugh, MELD

Introduction

Traditionally, liver biopsy is the reference method for the diagnosis of liver fibrosis and evaluation of its severity [1, 2]. However, its limitations are obvious in clinical practice [3, 4]. First, this procedure is invasive and potentially increases the risk of bleeding complications. Second, this procedure is expensive. Third, only 1/50000 of liver mass is obtained, which may result in the sampling error. Fourth, liver fibrosis progression is a dynamic process. Thus, the conditions of liver diseases should be repeatedly evaluated. However, the patients would poorly adhere to the repeated liver biopsies. Fifth, the intra-observer and inter-observer variations are significant in interpreting the histological examinations. Recently, non-invasive diagnostic methods have been developed [4-7]. Because liver fibrosis is characterized by the excessive deposition of extracellular matrix (ECM), serum markers which represent ECM components are

widely employed to assess the development of liver fibrosis [8-9]. Hyaluronic acid (HA), laminin (LN), amino-terminal pro-peptide of type III pro-collagen (PIIINP), and collagen IV (CIV) are four major serum markers of liver fibrosis. Until now, numerous studies have shown their potential clinical usefulness in the diagnosis of liver fibrosis and cirrhosis [10-19]. However, few study has explored the clinical significance of HA, LN, PIIINP, and CIV in patients with a diagnosis of liver cirrhosis. Herein, we have conducted a retrospective cross-sectional study to evaluate the correlations of the four serum liver fibrosis markers with the severity of liver dysfunction in cirrhotic patients.

Methods

Study design

Between January 2013 and June 2014, a total of 272 patients with a clinical diagnosis with liver cirrhosis were admitted to the General

Table 1. Characteristics of included patients

Variables	Values
Age (years)	56.99±0.77
Sex (Male/Female) - n.	156/72
RBC (10 ¹² /L)	3.18±0.05
Hb (g/L)	94.08±1.99
WBC (10 ⁹ /L)	4.94±0.25
PLT (10 ⁹ /L)	96.65±4.81
TBIL (umol/L)	41.74±4.25
ALB (g/L)	32.14±0.40
ALT (U/L)	56.62±9.04
AST (U/L)	76.07±9.12
ALP (U/L)	117.56±6.07
GGT (U/L)	132.25±22.78
BUN (mmol/L)	7.11±0.44
Cr (umol/L)	78.33±6.42
PT (seconds)	16.30±0.21
APTT (seconds)	43.14±0.53
INR	1.32±0.02
HA (ng/ml)	1554.44±629.73
LN (ng/ml)	158.76±8.25
PIIINP (ng/ml)	242.87±84.59
CIV (ng/ml)	176.16±12.32
MELD score	7.58±0.50
Child score	7.65±0.14
Child class (A/B/C) - n.	72/92/43

Abbreviations: RBC, red blood cell; Hb, hemoglobin; WBC, white blood cell; PLT, platelets count; TBIL, total bilirubin; ALB, albumin; ALT, alanine, aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transferase; BUN, blood urea nitrogen; Cr, creatinine; PT, prothrombin time; APTT, activated partial thromboplastin time; INR, international normalized ratio; HA, hyaluronic acid, LN, laminin; PIIINP, amino-terminal propeptide of type III procollagen; CIV, collagen IV; MELD, model for the end-stage of liver diseases.

Hospital of Shenyang Military Area and underwent the tests for four serum liver fibrosis markers (i.e., HA, LN, PIIINP, and CIV). Among them, 44 patients with malignancy were excluded (hepatocellular carcinoma, n=37; cholangiocarcinoma, n=1; thyroid carcinoma, n=2; breast cancer, n=2; renal carcinoma, n=1; rectal cancer, n=1). Thus, 228 patients were included in this retrospective cross-sectional study. This study was conceived by two investigators (Qi X. and Guo X.), and the study protocol was approved by the Medical Ethical Committee of the General Hospital of Shenyang Military Area (number k(2015)01). The informed consents from patients were waived.

A total of 3 ml fasting venous blood sample was obtained from every patient and then centrifuged. The diagnostic kits for the HA, LN, PIIINP, and CIV were purchased from the Autobio Diagnostics Co. Ltd. (Zhengzhou, Henan Province, China). The reference values were as follows: HA<120 ng/ml, LN<130 ng/ml, PIIINP<15 ng/ml, and CIV<95 ng/ml. Two laboratory investigators (Xia C. and Chen J.) performed these tests by magnetism particulate chemiluminescence immunoassay and recorded their values.

Electronic medical charts of these patients were retrospectively reviewed. All demographic data (age and sex), etiology of liver cirrhosis, clinical presentation, regular laboratory data, and severity of liver dysfunction (Child-Pugh and MELD score) were retrospectively collected. The regular laboratory data included red blood cell (RBC), hemoglobin (Hb), white blood cell (WBC), platelets count (PLT), total bilirubin (TBIL), albumin (ALB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), blood urea nitrogen (BUN), creatinine (Cr), prothrombin time (PT), activated partial thromboplastin time (APTT), and international normalized ratio (INR). The data collection was conducted by five investigators (Zhu C., Peng Y., Dai J., Zhang W. and Hou Y.). The data accuracy was validated by two investigators (Qi X. and Zhu C.).

Statistical analyses

Categorical and continuous variables were reported as the frequency and mean ± standard errors, respectively. Correlations of HA, LN, PIIINP, and CIV levels with demographic and laboratory data were evaluated by Pearson's Chi-square tests. Correlation coefficient r with 95% confidence interval (CI) was calculated (0<r<1, positive correlation; r=0, zero correlation; -1<r<0, negative correlation). The subgroup analyses were conducted according to the etiology of liver cirrhosis (hepatitis B virus infection alone/alcohol abuse alone). We also drew the boxplots to evaluate the differences of HA, LN, PIIINP, and CIV levels according to the Child-Pugh classes (A, B, and C). P<0.05 was considered statistically significant. All statistical analyses were performed by using the MedCalc software version 11.4.2.0.

HA, LN, PIIINP, and CIV in liver cirrhosis

Table 2. Correlation of four liver fibrosis markers with the demographic and laboratory data

Variables	HA		LN		PIIINP		CIV	
	Coefficient r (95% CI)	P	Coefficient r (95% CI)	P	Coefficient r (95% CI)	P	Coefficient r (95% CI)	P
Age	0.002921 (-0.1273 to 0.1331)	0.9651	-0.02050 (-0.1503 to 0.1100)	0.7587	0.05272 (-0.07803 to 0.1817)	0.4293	-0.07910 (-0.2072 to 0.05165)	0.2352
Sex (Male/Female)	-0.01183 (-0.1415 to 0.1183)	0.8590	-0.1729 (-0.2961 to -0.04393)	0.0089	0.06055 (-0.06993 to 0.1890)	0.3628	-0.1313 (-0.2569 to -0.001421)	0.0476
RBC	0.05615 (-0.07699 to 0.1873)	0.4083	-0.1196 (-0.2482 to 0.01320)	0.0774	-0.03084 (-0.1627 to 0.1022)	0.6499	-0.1312 (-0.2593 to 0.001391)	0.0525
Hb	-0.03671 (-0.1682 to 0.09603)	0.5881	-0.03050 (-0.1621 to 0.1022)	0.6528	0.03333 (-0.09938 to 0.1649)	0.6229	-0.03814 (-0.1696 to 0.09461)	0.5737
WBC	-0.01106 (-0.1434 to 0.1217)	0.8707	0.1495 (0.01722 to 0.2766)	0.0270	0.02727 (-0.1057 to 0.1593)	0.6882	0.3156 (0.1910 to 0.4302)	<0.0001
PLT	-0.02081 (-0.1530 to 0.1121)	0.7594	-0.02898 (-0.1609 to 0.1040)	0.6697	-0.009480 (-0.1419 to 0.1233)	0.8891	-0.02227 (-0.1544 to 0.1106)	0.7432
TBIL	0.08953 (-0.04117 to 0.2172)	0.1789	0.2071 (0.07900 to 0.3284)	0.0017	-0.01935 (-0.1492 to 0.1111)	0.7719	0.2664 (0.1411 to 0.3833)	<0.0001
ALB	-0.1308 (-0.2564 to -0.0008693)	0.0486	-0.1800 (-0.3029 to -0.05129)	0.0064	-0.1009 (-0.2279 to 0.02940)	0.1287	-0.1308 (-0.2564 to -0.0009215)	0.0485
ALT	-0.01474 (-0.1447 to 0.1157)	0.8252	0.003566 (-0.1267 to 0.1337)	0.9574	-0.03051 (-0.1601 to 0.1001)	0.6475	0.02614 (-0.1044 to 0.1558)	0.6952
AST	0.007857 (-0.1225 to 0.1379)	0.9063	0.07223 (0.05853 to 0.2006)	0.2785	-0.02039 (-0.1502 to 0.1101)	0.7600	0.08561 (-0.04510 to 0.2134)	0.1987
ALP	0.01366 (-0.1168 to 0.1436)	0.8378	0.02016 (-0.1103 to 0.1500)	0.7626	0.04218 (-0.08852 to 0.1715)	0.5272	-0.02359 (-0.1533 to 0.1070)	0.7237
GGT	-0.003593 (-0.1337 to 0.1267)	0.9571	0.07218 (-0.05859 to 0.2005)	0.2789	-0.01143 (-0.1414 to 0.1190)	0.8640	0.1079 (-0.02261 to 0.2348)	0.1049
BUN	-0.04929 (-0.1819 to 0.08508)	0.4722	0.1252 (-0.008716 to 0.2548)	0.0668	-0.0008079 (-0.1346 to 0.1330)	0.9906	0.05623 (-0.07816 to 0.1886)	0.4120
Cr	-0.03158 (-0.1647 to 0.1027)	0.6451	0.02124 (-0.1129 to 0.1546)	0.7568	-0.005846 (-0.1395 to 0.1281)	0.9321	0.01305 (-0.1210 to 0.1466)	0.8492
PT	0.1152 (-0.02052 to 0.2467)	0.0959	0.1795 (0.04521 to 0.3074)	0.0091	0.07536 (-0.06065 to 0.2086)	0.2770	0.2139 (0.08089 to 0.3395)	0.0018
APTT	-0.01154 (-0.1473 to 0.1247)	0.8686	0.1086 (-0.02787 to 0.2411)	0.1185	0.06623 (-0.07045 to 0.2005)	0.3419	0.09904 (-0.03750 to 0.2320)	0.1546
INR	0.1098 (-0.02664 to 0.2422)	0.1144	0.1820 (0.04717 to 0.3104)	0.0085	0.07480 (-0.06187 to 0.2087)	0.2829	0.2107 (0.07683 to 0.3371)	0.0023
MELD score	0.07926 (-0.05979 to 0.2153)	0.2633	0.2588 (0.1248 to 0.3834)	0.0002	0.04573 (-0.09325 to 0.1830)	0.5191	0.1795 (0.04216 to 0.3102)	0.0108
Child-Pugh score	0.1612 (0.02543 to 0.2912)	0.0203	0.2445 (0.1118 to 0.3685)	0.0004	0.02665 (-0.1101 to 0.1624)	0.7031	0.2361 (0.1031 to 0.3609)	0.0006

Abbreviations: RBC, red blood cell; Hb, hemoglobin; WBC, white blood cell; PLT, platelets count; TBIL, total bilirubin; ALB, albumin; ALT, alanine, aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transferase; BUN, blood urea nitrogen; Cr, creatinine; PT, prothrombin time; APTT, activated partial thromboplastin time; INR, international normalized ratio; HA, hyaluronic acid; LN, laminin; PIIINP, amino-terminal propeptide of type III procollagen; CIV, collagen IV; MELD, model for the end-stage of liver diseases.

HA, LN, PIIINP, and CIV in liver cirrhosis

Table 3. Correlation of LN with the demographic and laboratory data in male and female patients

Variables	LN in Male		LN in Female	
	Coefficient r (95% CI)	P	Coefficient r (95% CI)	P
Age	-0.03414 (-0.1903 to 0.1237)	0.6722	0.1798 (-0.05589 to 0.3964)	0.1336
RBC	-0.1177 (-0.2728 to 0.04336)	0.1514	-0.1936 (-0.4114 to 0.04519)	0.1110
Hb	0.7231 (-0.1886 to 0.1317)	0.7231	-0.1255 (-0.3501 to 0.1128)	0.3006
WBC	0.1719 (0.01194 to 0.3232)	0.0355	-0.1173 (-0.3444 to 0.1228)	0.3371
PLT	-0.02345 (-0.1830 to 0.1373)	0.7757	-0.02981 (-0.2646 to 0.2083)	0.8079
TBIL	0.2238 (0.06859 to 0.3685)	0.0051	0.1243 (-0.1105 to 0.3460)	0.2981
ALB	-0.1175 (-0.2697 to 0.04038)	0.1440	-0.4106 (-0.5864 to -0.1977)	0.0003
ALT	0.04572 (-0.1127 to 0.2019)	0.5722	-0.06623 (-0.2934 to 0.1680)	0.5804
AST	0.09995 (-0.05863 to 0.2536)	0.2159	0.04643 (-0.1873 to 0.2751)	0.6986
ALP	0.001123 (-0.1566 to 0.1587)	0.9889	0.1626 (-0.07174 to 0.3800)	0.1723
GGT	0.07218 (-0.08646 to 0.2272)	0.3721	0.009377 (-0.2228 to 0.2405)	0.9377
BUN	0.1226 (-0.04008 to 0.2790)	0.1390	-0.08061 (-0.3130 to 0.1609)	0.5135
Cr	-0.001853 (-0.1637 to 0.1601)	0.9822	-0.05900 (-0.2933 to 0.1820)	0.6327
PT	0.0283 (0.02007 to 0.3408)	0.0283	0.08369 (-0.1543 to 0.3125)	0.4909
APTT	0.04164 (-0.1251 to 0.2061)	0.6252	0.2866 (0.05173 to 0.4915)	0.0178
INR	0.1888 (0.02361 to 0.3439)	0.0255	0.07385 (-0.1675 to 0.3069)	0.5495
MELD score	0.2360 (0.07049 to 0.3889)	0.0057	0.1755 (-0.07144 to 0.4022)	0.1619
Child-Pugh score	0.2092 (0.04423 to 0.3631)	0.0135	0.3149 (0.08265 to 0.5147)	0.0089

Abbreviations: RBC, red blood cell; Hb, hemoglobin; WBC, white blood cell; PLT, platelets count; TBIL, total bilirubin; ALB, albumin; ALT, alanine, aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transferase; BUN, blood urea nitrogen; Cr, creatinine; PT, prothrombin time; APTT, activated partial thromboplastin time; INR, international normalized ratio; HA, hyaluronic acid, LN, laminin; PIIINP, amino-terminal propeptide of type III procollagen; CIV, collagen IV; MELD, model for the end-stage of liver diseases.

Results

Characteristics

The characteristics of patients at their admissions were shown in **Table 1**. A majority of patients were male. The etiology of liver cirrhosis included hepatitis B virus infection (n=62), hepatitis C virus infection (n=14), a combination of hepatitis B and C virus infection (n=2), alcohol abuse alone (n=77), a combination of hepatitis B virus infection and alcohol abuse (n=7), a combination of hepatitis C virus infection and alcohol abuse (n=2), autoimmune liver diseases (n=7), drug-related liver diseases (n=9), primary biliary cirrhosis (n=4), schistosome (n=1), and unknown (n=43). Of them, 32%, 40% and 18% had Child-Pugh class A, B, and C, respectively.

Correlations in all patients

Correlations of HA, LN, PIIINP, and CIV levels with demographic and laboratory data were demonstrated in **Table 2**.

HA level significantly correlated with ALB (coefficient r: -0.1308, P=0.0486) and Child-Pugh score (coefficient r: 0.1612, P=0.0203), but not with TBIL, PT, INR, or MELD score.

LN level significantly correlated with sex (coefficient r: -0.1729, P=0.0089), WBC (coefficient r: 0.1495, P=0.0270), TBIL (coefficient r: 0.2071, P=0.0017), ALB (coefficient r: -0.1800, P=0.0064), PT (coefficient r: 0.1795, P=0.0091), INR (coefficient r: 0.1820, P=0.0085), MELD score (coefficient r: 0.2588, P=0.0002), and Child-Pugh score (coefficient r: 0.2445, P=0.0004). Given the correlation of LN level with sex, a subgroup analysis was performed according to the sexes (male and female). The results were shown in **Table 3**. LN level significantly correlated with Child-Pugh score in both male and female patients. However, its significant correlation with MELD score was just maintained in the male subgroup.

PIIINP level did not significantly correlate with any markers of liver function or Child-Pugh/MELD scores.

HA, LN, PIIINP, and CIV in liver cirrhosis

Table 4. Correlation of CIV with the demographic and laboratory data in male and female patients

Variables	CIV in Male		CIV in Female	
	Coefficient r (95% CI)	P	Coefficient r (95% CI)	P
Age	-0.05706 (-0.2123 to 0.1010)	0.4792	-0.03961 (-0.2704 to 0.1955)	0.4792
RBC	-0.1567 (-0.3092 to 0.003624)	0.0554	-0.05874 (-0.2914 to 0.1805)	0.6316
Hb	-0.03355 (-0.1928 to 0.1274)	0.6836	0.01135 (-0.2242 to 0.2457)	0.9257
WBC	0.3580 (0.2098 to 0.4901)	<0.0001	-0.1247 (-0.3510 to 0.1154)	0.3072
PLT	0.01857 (-0.1421 to 0.1783)	0.8215	-0.1548 (-0.3777 to 0.08499)	0.2041
TBIL	0.2641 (0.1110 to 0.4049)	0.0009	0.0229 (0.03860 to 0.4704)	0.0229
ALB	-0.1067 (-0.2595 to 0.05129)	0.1849	-0.2266 (-0.4354 to 0.005330)	0.0556
ALT	-0.008091 (-0.1655 to 0.1498)	0.9204	0.1494 (-0.08525 to 0.3683)	0.2105
AST	0.03007 (-0.1282 to 0.1868)	0.7104	0.2917 (0.06440 to 0.4903)	0.0129
ALP	0.06281 (-0.09579 to 0.2183)	0.4375	-0.08124 (-0.3071 to 0.1533)	0.4975
GGT	0.1004 (-0.05819 to 0.2540)	0.2139	0.1502 (-0.08445 to 0.3690)	0.2080
BUN	0.05173 (-0.1111 to 0.2119)	0.5338	-0.1269 (-0.3546 to 0.1150)	0.3025
Cr	0.004468 (-0.1575 to 0.1662)	0.9572	-0.2007 (-0.4191 to 0.03964)	0.1008
PT	0.1867 (0.02146 to 0.3420)	0.0272	0.2635 (0.03045 to 0.4694)	0.0275
APTT	0.03783 (-0.1289 to 0.2025)	0.6572	0.3052 (0.07204 to 0.5068)	0.0114
INR	0.1808 (0.01537 to 0.3366)	0.0325	0.2742 (0.03832 to 0.4812)	0.0236
MELD score	0.1546 (-0.01408 to 0.3148)	0.0723	0.1565 (-0.09091 to 0.3857)	0.2133
Child-Pugh score	0.2256 (0.06144 to 0.3779)	0.0076	0.2407 (0.002434 to 0.4531)	0.0480

Abbreviations: RBC, red blood cell; Hb, hemoglobin; WBC, white blood cell; PLT, platelets count; TBIL, total bilirubin; ALB, albumin; ALT, alanine, aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamine transferase; BUN, blood urea nitrogen; Cr, creatinine; PT, prothrombin time; APTT, activated partial thromboplastin time; INR, international normalized ratio; HA, hyaluronic acid, LN, laminin; PIIINP, amino-terminal propeptide of type III procollagen; CIV, collagen IV; MELD, model for the end-stage of liver diseases.

CIV level significantly correlated with sex (coefficient r: -0.1313, P=0.0476), WBC (coefficient r: 0.3156, P<0.0001), TBIL (coefficient r: 0.2664, P<0.0001), ALB (coefficient r: -0.1308, P=0.0485), PT (coefficient r: 0.2139, P=0.0018), INR (coefficient r: 0.2107, P=0.0023), MELD score (coefficient r: 0.1795, P=0.0108), and Child-Pugh score (coefficient r: 0.2361, P=0.0006). Given the correlation of CIV level with sex, a subgroup analysis was performed according to the sexes (male and female). The results were shown in **Table 4**. CIV level significantly correlated with Child-Pugh score in both male and female patients. However, the significant correlation with MELD score disappeared in the two sex subgroups.

Correlations in patients with hepatitis B virus infection alone related liver cirrhosis

Correlations of HA, LN, PIIINP, and CIV levels with demographic and laboratory data in patients with hepatitis B virus infection alone related liver cirrhosis were demonstrated in **Table 5**.

HA level significantly correlated with sex (coefficient r: 0.3193, P=0.0114) and ALP (coefficient r: 0.3882, P=0.0018), but not with TBIL, ALB, PT, INR, or Child-Pugh/MELD score.

LN level significantly correlated with TBIL (coefficient r: 0.2797, P=0.0277), ALT (coefficient r: 0.2519, P=0.0483), AST (coefficient r: 0.2858, P=0.0243), GGT (coefficient r: 0.4176, P=0.0007), PT (coefficient r: 0.2931, P=0.0283), APTT (coefficient r: 0.2709, P=0.0476), and INR (coefficient r: 0.2845, P=0.0371), but not with ALB or Child-Pugh/MELD score.

PIIINP level significantly correlated with PT (coefficient r: 0.2982, P=0.0256), APTT (coefficient r: 0.2703, P=0.0480), and INR (coefficient r: 0.3026, P=0.0262), but not with TBIL, ALB, or Child-Pugh/MELD score.

CIV level significantly correlated with TBIL (coefficient r: 0.4051, P=0.0011), ALB (coefficient r: -0.3133, P=0.0132), AST (coefficient r: 0.2581, P=0.0428), PT (coefficient r: 0.3034, P=0.0230), APTT (coefficient r: 0.4462, P=

HA, LN, PIIINP, and CIV in liver cirrhosis

Table 5. Correlation of four liver fibrosis markers with the demographic and laboratory data in patients with hepatitis virus infection alone related liver cirrhosis

Variables	HA		LN		PIIINP		CIV	
	Coefficient r (95% CI)	P	Coefficient r (95% CI)	P	Coefficient r (95% CI)	P	Coefficient r (95% CI)	P
Age	0.1722 (-0.08111 to 0.4045)	0.1809	-0.005203 (-0.2546 to 0.2449)	0.9680	0.02969 (-0.2217 to 0.2774)	0.8188	0.1476 (-0.1061 to 0.3832)	0.2523
Sex (Male/Female)	0.3193 (0.07559 to 0.5271)	0.0114	-0.04956 (-0.2957 to 0.2027)	0.7020	-0.06175 (-0.3068 to 0.1910)	0.6335	-0.05413 (-0.2998 to 0.1983)	0.6761
RBC	-0.06863 (-0.3191 to 0.1908)	0.6055	-0.04269 (-0.2955 to 0.2158)	0.7482	0.1157 (-0.1447 to 0.3611)	0.3828	-0.06197 (-0.3131 to 0.1972)	0.6410
Hb	0.04029 (-0.2180 to 0.2933)	0.7619	0.02661 (-0.2311 to 0.2808)	0.8414	0.1449 (-0.1154 to 0.3867)	0.2734	0.02670 (-0.2310 to 0.2809)	0.8409
WBC	-0.03078 (-0.2846 to 0.2271)	0.8170	0.1113 (-0.1491 to 0.3572)	0.4015	-0.07731 (-0.3269 to 0.1824)	0.5606	0.2559 (-0.0001739 to 0.4805)	0.0504
PLT	-0.1490 (-0.3902 to 0.1114)	0.2601	-0.1555 (-0.3958 to 0.1047)	0.2396	-0.1904 (-0.4258 to 0.06901)	0.1485	-0.2158 (-0.4471 to 0.04269)	0.1008
TBIL	0.03169 (-0.2198 to 0.2793)	0.8068	0.2797 (0.03214 to 0.4949)	0.0277	-0.01374 (-0.2626 to 0.2368)	0.9156	0.4051 (0.1729 to 0.5947)	0.0011
ALB	-0.1037 (-0.3446 to 0.1499)	0.4224	-0.1800 (-0.4263 to 0.05491)	0.1237	-0.08154 (-0.3247 to 0.1717)	0.5287	-0.3133 (-0.5222 to -0.06889)	0.0132
ALT	0.03951 (-0.2124 to 0.2865)	0.7604	0.2519 (0.002231 to 0.4719)	0.0483	-0.01526 (-0.2640 to 0.2354)	0.9063	0.1384 (-0.1154 to 0.3752)	0.2834
AST	0.1078 (-0.1459 to 0.3482)	0.4043	0.2858 (0.03882 to 0.4999)	0.0243	-0.02384 (-0.2720 to 0.2273)	0.8540	0.2581 (0.008926 to 0.4771)	0.0428
ALP	0.3882 (0.1533 to 0.5816)	0.0018	0.1098 (-0.1440 to 0.3499)	0.3957	0.03500 (-0.2167 to 0.2823)	0.7871	0.03346 (-0.2181 to 0.2809)	0.7963
GGT	0.1172 (-0.1366 to 0.3565)	0.3645	0.4176 (0.1874 to 0.6043)	0.0007	0.2274 (-0.02371 to 0.4515)	0.0755	0.07320 (-0.1799 to 0.3172)	0.5718
BUN	-0.04320 (-0.3027 to 0.2222)	0.7519	-0.1377 (-0.3866 to 0.1299)	0.3115	0.01968 (-0.2445 to 0.2811)	0.8856	0.1066 (-0.1608 to 0.3595)	0.4341
Cr	-0.06628 (-0.3235 to 0.2001)	0.6274	-0.1481 (-0.3956 to 0.1194)	0.2759	0.02131 (-0.2430 to 0.2826)	0.8761	0.1143 (-0.1533 to 0.3662)	0.4018
PT	0.1193 (-0.1482 to 0.3706)	0.3810	0.2931 (0.03275 to 0.5163)	0.0283	0.2982 (0.03831 to 0.5203)	0.0256	0.3034 (0.04396 to 0.5244)	0.0230
APTT	-0.0003901 (-0.2681 to 0.2674)	0.9978	0.2709 (0.003380 to 0.5022)	0.0476	0.2703 (0.002764 to 0.5018)	0.0480	0.4462 (0.2026 to 0.6377)	0.0007
INR	0.1069 (-0.1656 to 0.3642)	0.4417	0.2845 (0.01807 to 0.5131)	0.0371	0.3026 (0.03789 to 0.5276)	0.0262	0.287 (0.02089 to 0.5152)	0.0353
MELD score	0.01340 (-0.2632 to 0.2879)	0.9256	0.2050 (-0.07482 to 0.4549)	0.1490	0.1399 (-0.1411 to 0.4001)	0.3275	0.3240 (0.05321 to 0.5505)	0.0204
Child-Pugh score	0.1115 (-0.1611 to 0.3683)	0.4221	0.2391 (-0.03059 to 0.4764)	0.0816	0.1952 (-0.07660 to 0.4399)	0.1573	0.3376 (0.07678 to 0.5552)	0.0125

Abbreviations: RBC, red blood cell; Hb, hemoglobin; WBC, white blood cell; PLT, platelets count; TBIL, total bilirubin; ALB, albumin; ALT, alanine, aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transferase; BUN, blood urea nitrogen; Cr, creatinine; PT, prothrombin time; APTT, activated partial thromboplastin time; INR, international normalized ratio; HA, hyaluronic acid; LN, laminin; PIIINP, amino-terminal propeptide of type III procollagen; CIV, collagen IV; MELD, model for the end-stage of liver diseases.

0.0007), INR (coefficient r : 0.287, $P=0.0353$), MELD score (coefficient r : 0.3240, $P=0.0204$), and Child-Pugh score (coefficient r : 0.3376, $P=0.0125$).

Correlations in patients with alcohol abuse alone related liver cirrhosis

Correlations of HA, LN, PIIINP, and CIV levels with demographic and laboratory data in patients with alcohol abuse alone related liver cirrhosis were demonstrated in **Table 6**.

HA level did not significantly correlate with any markers of liver function or Child-Pugh/MELD scores.

LN level significantly correlated with WBC (coefficient r : 0.2677, $P=0.0211$), BUN (coefficient r : 0.3236, $P=0.0059$), and MELD score (coefficient r : 0.2561, $P=0.0395$), but not with TBIL, ALB, PT, INR, or Child-Pugh score.

PIIINP level significantly correlated with sex (coefficient r : 0.4551, $P<0.0001$) and ALP (coefficient r : 0.2571, $P=0.0250$), but not with TBIL, ALB, PT, INR, or Child-Pugh/MELD score.

CIV level significantly correlated with TBIL (coefficient r : 0.3101, $P=0.0064$), but not with any other markers of liver function or Child-Pugh/MELD scores.

Comparison among the different Child-Pugh classes

HA, LN, PIIINP, and CIV levels were compared among the patients with different Child-Pugh classes (**Figure 1**). All of the four serum liver fibrosis markers were higher in patients with Child-Pugh class B or C than in those with Child-Pugh class A.

Discussion

Child-Pugh score/class is the first prognostic index widely employed for liver cirrhosis [20]. It includes five variables (i.e., TBIL, ALB, PT or INR, hepatic encephalopathy, and ascites). The survival of cirrhotic patients is reduced, if Child-Pugh scores/classes are increased [21]. We found that HA, LN, and CIV levels significantly correlated with Child-Pugh scores. This finding suggested that the three serum markers might be indirectly associated with the survival of liver cirrhosis. It could be explained by the fact that the serum markers of liver fibrosis reflect

the severity of liver fibrosis and correlate with one or more components of Child-Pugh scoring system. Additionally, MELD score is an important prognostic index for liver cirrhosis [22, 23], which is used as a criterion for liver transplant candidates to allocate the liver donor in the USA [24]. It includes three laboratory variables (i.e., TBIL, INR, and Cr). In consistency with the Child-Pugh score, the survival of cirrhotic patients is inversely related to the MELD scores. We found that only LN and CIV levels significantly correlated with MELD scores, rather than HA or PIIINP level. This might be because the serum liver fibrosis markers just correlate with the liver function variables (i.e., TBIL and/or INR), but not reflect the renal function variables (i.e., Cr level). However, it should be noted that the positive correlations of serum liver fibrosis markers with Child-Pugh and MELD scores were weak, because all of their coefficient r values were less than 0.3. Therefore, our findings should be cautiously interpreted.

Hepatitis C virus infection is a major cause of liver cirrhosis in West [25]. By comparison, hepatitis B virus infection is the most frequent etiology of liver cirrhosis in China [26, 27]. In Northeastern China (Liaoning, Jilin, and Heilongjiang provinces), alcohol abuse is another important etiology [27]. In the subgroup analyses, we also paid more attention to the potential values of the four serum markers of liver fibrosis in patients with liver cirrhosis secondary to hepatitis B virus infection or alcohol abuse alone. As for the patients with liver cirrhosis secondary to hepatitis B virus infection alone, we found the following. 1) Both LN and CIV levels were significantly associated with TBIL and INR, but not HA or PIIINP level. 2) CIV level was also significantly associated with ALB and Child-Pugh score. 3) Although the correlation of LN level with Child-Pugh score was not significant, the P value was close to 0.05. Accordingly, it could be concluded that LN and CIV levels might be associated with the severity of liver dysfunction in patients with liver cirrhosis secondary to hepatitis B virus infection alone. On the other hand, as for the patients with liver cirrhosis secondary to alcohol abuse alone, we found that all of the four serum liver fibrosis markers were not significantly associated with Child-Pugh score. Accordingly, it could be concluded that HA, LN, PIIINP, and CIV levels might not be appropriate to reflect the severity

HA, LN, PIIINP, and CIV in liver cirrhosis

Table 6. Correlation of four liver fibrosis markers with the demographic and laboratory data in patients with alcohol alone related liver cirrhosis

Variables	HA		LN		PIIINP		CIV	
	Coefficient r (95% CI)	P	Coefficient r (95% CI)	P	Coefficient r (95% CI)	P	Coefficient r (95% CI)	P
Age	0.007478 (-0.2169 to 0.2311)	0.9485	-0.01398 (-0.2372 to 0.2107)	0.9040	0.08981 (-0.1369 to 0.3076)	0.4373	-0.1244 (-0.3389 to 0.1025)	0.2812
Sex (Male/Female)	0.08629 (-0.1404 to 0.3044)	0.4555	-0.1789 (-0.3874 to 0.04697)	0.1195	0.4551 (0.2574 to 0.6163)	<0.0001	-0.05589 (-0.2764 to 0.1702)	0.6292
RBC	0.1506 (-0.08066 to 0.3665)	0.2002	-0.2268 (-0.4329 to 0.001798)	0.0520	-0.04687 (-0.2725 to 0.1836)	0.6917	-0.2194 (-0.4265 to 0.009614)	0.0604
Hb	-0.06026 (-0.2848 to 0.1706)	0.6100	-0.1082 (-0.3286 to 0.1234)	0.3589	0.03931 (-0.1909 to 0.2654)	0.7395	-0.07448 (-0.2979 to 0.1567)	0.5282
WBC	-0.04190 (-0.2678 to 0.1884)	0.7230	0.2677 (0.04178 to 0.4676)	0.0211	-0.02110 (-0.2484 to 0.2084)	0.8584	-0.04190 (-0.2678 to 0.1884)	0.7230
PLT	-0.04013 (-0.2662 to 0.1901)	0.7342	0.05656 (-0.1742 to 0.2814)	0.6322	-0.05430 (-0.2793 to 0.1764)	0.6459	0.1042 (-0.1274 to 0.3249)	0.3770
TBIL	0.09001 (-0.1383 to 0.3092)	0.4394	0.1667 (-0.06101 to 0.3780)	0.1500	-0.03775 (-0.2610 to 0.1893)	0.7461	0.3101 (0.09096 to 0.5005)	0.0064
ALB	-0.1939 (-0.4005 to 0.03149)	0.0912	-0.1965 (-0.4027 to 0.02879)	0.0868	-0.1428 (-0.3554 to 0.08389)	0.2154	-0.01730 (-0.2403 to 0.2075)	0.8813
ALT	-0.02747 (-0.2514 to 0.1992)	0.8138	-0.05375 (-0.2759 to 0.1738)	0.6447	-0.03508 (-0.2585 to 0.1919)	0.7636	0.01068 (-0.2153 to 0.2356)	0.9270
AST	0.01373 (-0.2124 to 0.2385)	0.9063	-0.03083 (-0.2545 to 0.1960)	0.7915	-0.01958 (-0.2440 to 0.2068)	0.8667	0.06819 (-0.1597 to 0.2892)	0.5583
ALP	0.02900 (-0.1978 to 0.2528)	0.8036	-0.07939 (-0.2995 to 0.1487)	0.4954	0.2571 (0.03354 to 0.4561)	0.0250	0.05085 (-0.1766 to 0.2732)	0.6627
GGT	-0.02201 (-0.2462 to 0.2045)	0.8503	-0.009725 (-0.2347 to 0.2162)	0.9336	-0.03095 (-0.2546 to 0.1959)	0.7907	0.1044 (-0.1240 to 0.3223)	0.3694
BUN	-0.07408 (-0.3022 to 0.1620)	0.5392	0.3236 (0.09768 to 0.5178)	0.0059	-0.04224 (-0.2729 to 0.1930)	0.7265	0.04056 (-0.1946 to 0.2713)	0.7370
Cr	-0.08621 (-0.3132 to 0.1501)	0.4747	0.2187 (-0.01538 to 0.4301)	0.0669	-0.09062 (-0.3172 to 0.1458)	0.4523	-0.04646 (-0.2768 to 0.1889)	0.7004
PT	0.1313 (-0.1106 to 0.3585)	0.2860	0.1313 (-0.1106 to 0.3585)	0.2860	0.08338 (-0.1582 to 0.3155)	0.4991	0.1782 (-0.06293 to 0.3996)	0.1460
APTT	-0.04070 (-0.2764 to 0.1997)	0.7417	-0.001235 (-0.2396 to 0.2373)	0.9920	0.1049 (-0.1369 to 0.3350)	0.3945	-0.01498 (-0.2525 to 0.2243)	0.9035
INR	0.1121 (-0.1298 to 0.3414)	0.3629	0.1396 (-0.1022 to 0.3659)	0.2562	0.07914 (-0.1624 to 0.3117)	0.5212	0.1733 (-0.06793 to 0.3954)	0.1576
MELD score	0.05562 (-0.1909 to 0.2955)	0.6599	0.2561 (0.01299 to 0.4706)	0.0395	-0.01203 (-0.2552 to 0.2326)	0.9242	0.1544 (-0.09301 to 0.3838)	0.2194
Child-Pugh score	0.1852 (-0.05754 to 0.4073)	0.1334	0.1819 (-0.06098 to 0.4044)	0.1407	-0.02194 (-0.2608 to 0.2194)	0.8601	0.2056 (-0.03640 to 0.4248)	0.0951

Abbreviations: RBC, red blood cell; Hb, hemoglobin; WBC, white blood cell; PLT, platelets count; TBIL, total bilirubin; ALB, albumin; ALT, alanine, aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamine transferase; BUN, blood urea nitrogen; Cr, creatinine; PT, prothrombin time; APTT, activated partial thromboplastin time; INR, international normalized ratio; HA, hyaluronic acid; LN, laminin; PIIINP, amino-terminal propeptide of type III procollagen; CIV, collagen IV; MELD, model for the end-stage of liver diseases.

HA, LN, PIIINP, and CIV in liver cirrhosis

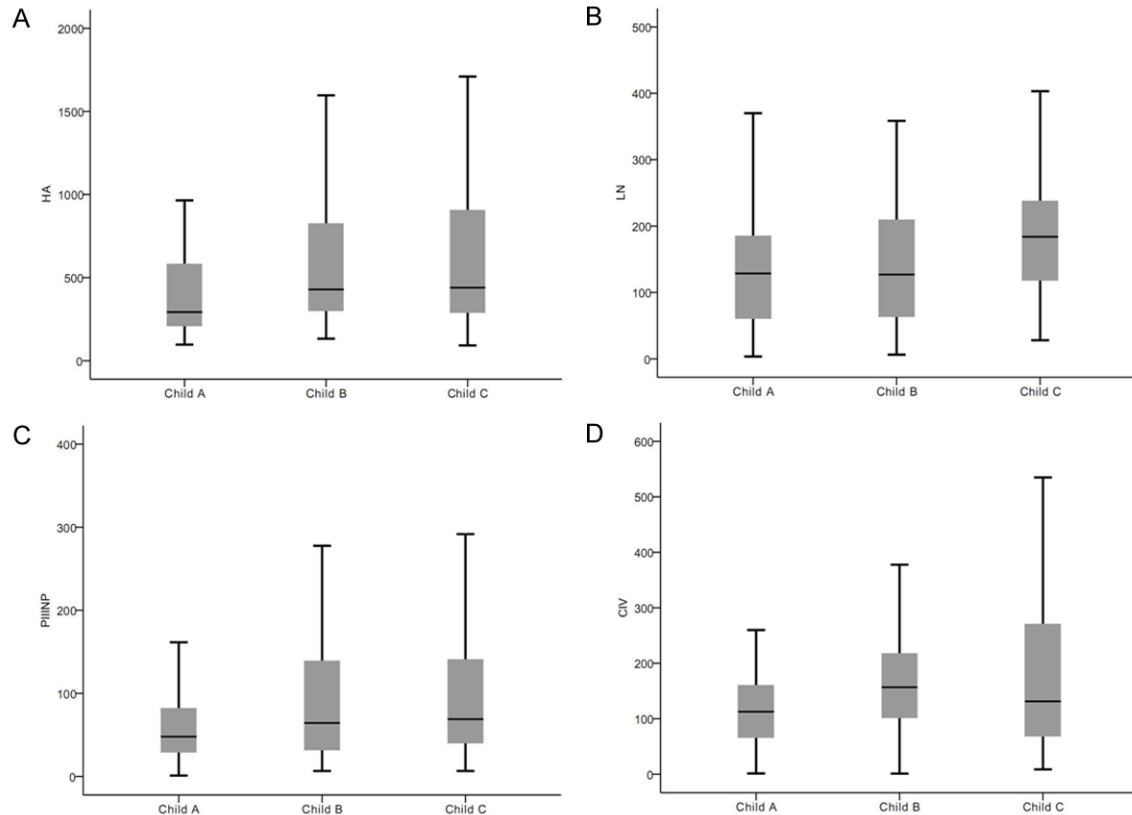


Figure 1. Boxplots of HA, LN, PIIINP, and CIV levels according to the Child-Pugh classes.

of liver dysfunction in patients with liver cirrhosis secondary to alcohol abuse alone.

This study had several limitations. First, this was a preliminary study, in which only Child-Pugh and MELD scores, two common scoring systems for the prognosis of liver cirrhosis, were evaluated. Further studies should be necessary to evaluate some clinically hard endpoints, such as death and hepatic decompensation. Second, this was a retrospective study, in which not all consecutive patients underwent the tests for serum liver fibrosis markers, thereby potentially resulting in the bias of patient selection. Third, the interobserver variability should be acknowledged, despite a standard technical procedure has been developed.

In conclusions, our study demonstrated the clinical utilities HA, LN, and CIV levels in reflecting the severity of liver dysfunction in patients with liver cirrhosis. However, given that their correlations were weak, prospective validations should be warranted. Additionally, it appeared that serum liver fibrosis markers should be

more likely to be valuable in patients with liver cirrhosis secondary to hepatitis B virus alone, but not in patients with liver cirrhosis secondary to alcohol abuse alone.

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XQ and XG designed the work. XQ also performed the statistical analyses and wrote the draft. CZ, YP, JD, WZ, and YH participated in the data collection. JC and CX participated in the tests of serum liver fibrosis markers. HL and XG gave the critical comments for this work.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Xiaozhong Guo and Xingshun Qi, Department of Gastroenterology, General Hospital of Shenyang Military Area, 83 Wenhua Road, Shenyang 110840, China. Tel: 86-24-28897603; Fax: 86-24-28851113; E-mail: guo_xiao_zhong@126.com (XZG); xingshunqi@126.com (XSQ)

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