HEPATOTOLOGY

Correlation between serum galectin-9 levels and liver fibrosis

Koji Fujita,* Toshiro Niki,† Takako Nomura,* Kyoko Oura,* Tomoko Tadokoro,* Teppei Sakamoto,* Joji Tani,* Hirohito Yoneyama,* Asahiro Morishita,* Noriyuki Kuroda,‡ Takeshi Arai,§ Naoki Nishimoto,¶ Takashi Himoto,† Mitsuomi Hirashima† and Tsutomu Masaki*†

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Key words
chronic hepatitis, enzyme-linked immunosorbent assay, galectin-9, hepatocellular carcinoma, liver cirrhosis.

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Declaration of conflict of interest: Toshiro Niki and Mitsuomi Hirashima work for GalPharma Co., which is developing diagnostic and therapeutic applications of galectin-9; both were masked to clinical data until after analyses were completed. The remaining authors have no conflicts to disclose.

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Abstract

Background and Aim: Chronic liver diseases progress from chronic inflammation to fibrosis to tumorigenesis. Galectin-9, a β-galactoside-specific animal lectin, is indicated to contribute to all three steps of progression. The aim of this study was to determine which of the three steps was most dominant in elevating the serum galectin-9 concentration and to test the possibility of galectin-9 as a serum biomarker.

Methods: Japanese patients with chronic hepatitis, liver cirrhosis, hepatocellular carcinoma (HCC), non-alcoholic fatty liver disease, or alcoholic liver disease who provided informed consent were enrolled in this study. Serum galectin-9 levels were measured using a sandwich ELISA. Multiple regression analyses were performed using EZR to identify factors that determined serum galectin-9 concentration.

Results: One hundred one patients with 50 of chronic hepatitis and 51 of liver cirrhosis were enrolled; the cohort included 45 cases of hepatitis C virus infection, 13 cases of hepatitis B virus infection, and 46 cases with HCC-related complications. The median serum galectin-9 concentration was 77.54 pg/mL (interquartile range: 18.89–241.9 pg/mL). Multiple linear regression analyses proved Fibrosis-4 index and aspartate aminotransferase to platelet ratio index, indexes of liver fibrosis, were able to predict the serum galectin-9 levels with statistical significance. A multiple logistic regression analysis determined 10 pg/mL increase in the serum galectin-9 concentration presented an odds ratio of 3.90 for liver fibrosis progression.

Conclusions: The serum galectin-9 concentration represents a potential biomarker of liver fibrosis in patients with chronic liver diseases, regardless of chronic inflammation or the presence of HCC complications. Furthermore, higher serum galectin-9 levels are a predictor for liver fibrosis progression.

Introduction

Chronic liver diseases (CLDs) generally progress from inflammation to fibrosis and finally carcinogenesis.1 Hepatitis C virus (HCV) and B virus (HBV) account for a large percentage of chronic hepatitis etiologies,2 with approximately 2.8% of the world population infected with HCV.3 Regarding HBV, the global prevalence of HBV infection remains 3.7% despite widespread vaccination even in low income regions.4 The prevalence of diabetes mellitus, an independent risk factor of hepatocellular carcinoma (HCC), has been estimated to increase up to 69% in developing countries and 20% in developed countries between 2010 and 2030.5,6

Chronic liver diseases initially present chronic liver inflammation that eventually progresses to liver fibrosis and carcinogenesis. Galectin-9, a tandem repeat-type soluble animal lectin that specifically binds to β-galactosides, is implied to play pivotal roles during all three steps of CLDs progression. In case of inflammation, galectin-9 regulates T helper type 1 immunity by binding to its ligand, Tim-3.7 In chronic HCV infection, hepatic Kupffer cell-derived galectin-9 modulates T-cell immunity.8 The Tim-3/galectin-9 axis also causes T-cell exhaustion in chronic HBV infection.9 In the context of tumor immunity, this pathway is involved in HBV-related HCC and contributes to poor prognosis of HCC via T-cell senescence.10 Galectin-9 also plays crucial roles in tumor biology, which might support galectin-9 as a preventive factor of HCC. Intracellular galectin-9 expression is decreased compared with the levels in adjacent normal tissues and is closely related to pathological differentiation, TNM staging, and recurrence.11 Patients with galectin-9-positive HCC had a better prognosis than individuals with galectin-9-negative HCC.12 To mention galectin-9’s commitment to liver fibrosis, galectin family

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including galectin-9 is thought to connect cell surface to extracellular matrix.13

Thus, circulating galectin-9 has been proposed as a biomarker of CLDs. To the best of our knowledge, the one of earliest reports examining the circulating galectin-9 levels revealed individuals with chronic HCV infection presented higher galectin-9 values than healthy control subjects.8 However, galectin-9 is expressed during all three stages of liver disease: inflammation, fibrosis, and carcinogenesis, the latter of which can result in tumor immunity. Furthermore, chronic inflammation, fibrosis, and HCC coexist in a variety of clinical settings. Therefore, circulating galectin-9 levels should be variable during these three stages. The purpose of this study is to identify which factors relating to inflammation, fibrosis, and HCC determine the circulating galectin-9 levels and to elucidate the possibility of galectin-9 as a serum biomarker for CLDs.

Methods

Patients. This study was conducted in accordance with the ethical principles of the Declaration of Helsinki and was approved by the Institutional Review Board at Kagawa University, Faculty of Medicine. Japanese patients with chronic hepatitis (CH), liver cirrhosis (LC), and HCC, who agreed to preserve their serum between 2004 and 2013, were enrolled. Cirrhotic stage was clinically determined based on morphological changes of a liver figured by computed tomography or ultrasound sonography examinations and extrathepatic complications including esophageal or gastric varix and ascites. HCC was pointed out by computed tomography exams and tumor markers. HBV and HCV infection were determined by serological examinations. Autoimmune hepatitis and primary biliary cholangitis were clarified by liver biopsy examinations and serological tests. Non-alcoholic fatty liver disease and alcoholic liver disease were diagnosed by history of alcoholic consumption and existence of fatty liver pointed out through ultrasound sonography examinations. When this study was initiated, written informed consent was obtained to allow us to measure the serum galectin-9 levels in the samples. For patients who died and had no relatives listed in his or her clinical records, we provided opt-out methods for the relatives of the dead participants by publishing a summary of this study on our university website.14,15

Measurement of serum galectin-9 levels. The serum galectin-9 levels were determined using a previously described sandwich ELISA.8,10,17 In brief, 96-well plates (Nunc, Naperville, IL) were coated with an antihuman galectin-9 monoclonal antibody (9S2-3, BioLegend, San Diego, CA), blocked with 3% fetal bovine serum, and then incubated for 1 h with eightfold-diluted serum. After several washings, galectin-9 remaining in the wells was recognized by polyclonal antihuman galectin-9 antibody (Galpharma, Kagawa, Japan) conjugated with biotin and streptavidin-conjugated horseradish peroxidase (Invitrogen, Tokyo, Japan) plus the colorimetric substrate tetramethylbenzidine (KPL, Gaithersburg, MD). The optical density was read with a microplate spectrophotometer Bio-Rad (Hercules, CA). Quantification was performed using a standard curve constructed with known concentration of recombinant galectin-9 (Galpharma) diluted with eightfold-diluted normal human serum from a volunteer whose galectin-9 concentration is at the detection limit of the ELISA at this dilution.

Clinical parameter. The following clinical parameters were obtained from the participants’ medical records: platelet count (Plt), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin (T-Bil), serum creatinine (Cr), prothrombin time (PT and PT-international normalized ratio [PT-INR]), serum albumin, and Mac-2 binding protein glycosylation isomer (M2BPGi). M2BPGi, a liver fibrosis marker, was measured using a lectin-antibody sandwich immunoassay using HISCL-2000i (Sysmex, Hyogo, Japan). HbA1c as described by The Japan Diabetes Society was converted to values based on the NGSP.18 Fibrosis-4 (Fib-4) index was calculated using the following equation: age × AST (U/L)/Plt (10^9/L) × √ALT (U/L).19 Another index of liver fibrosis, AST to platelet ratio index (APRI), was calculated using the following equation: 100 × (AST (U/L)/Plt (10^9/L)).20 In our hospital, 35 U/L was applied as the upper limit of the normal AST values. The estimated glomerular filtration rate (eGFR) was calculated using the following equation: eGFR (mL/min/1.73 m^2) = 194 × Cr^-1.094 × age^-0.287 in males and eGFR (mL/min/1.73 m^2) = 194 × Cr^-1.094 × age^-0.287 × 0.739 in females.21

Statistical analysis. The serum galectin-9 levels and other continuous data among the clinical parameters are presented as median and interquartile range. Correlation analyses were conducted between galectin-9 and the continuous parameters using Spearman’s rank correlation coefficient, whereas Mann–Whitney U-test was applied to categorical parameters. Multiple linear regression analyses were performed to identify variables to predict serum galectin-9 levels. A multiple logistic regression analysis was performed to determine the odds ratio of higher serum galectin-9 concentrations for liver fibrosis. P values less than 0.05 were considered statistically significant. The optimized model was selected with the Akaike information criterion using EZR (Jichi Medical University, Saitama, Japan), a graphical user interface for R software (The R Foundation for Statistical Computing).22,23 A priori sample size calculation and post-hoc power test were performed using Free Statistics Calculators supplied by Daniel Soper (http://www.danielsoper.com/statcalc/default.aspx).

Results

Sample size calculation. The sample size was calculated using Free Statistics Calculators under the following conditions: anticipated effect size (f^2) = 0.15; desired statistical power level = 0.8; number of predictors = 3; and probability level = 0.05. The reason why three predictors were premised in the analysis was because galectin-9 is associated with three pathological mechanisms in CLDs (i.e., inflammation, fibrosis, and carcinoma), with hypothesis that ALT, Fib-4 index, and HCC complication might predict serum galectin-9 concentration. Thus, the minimum required sample size calculated was 76 cases.
**Demographic and clinicopathological characteristics of the patients.** A total of 101 patients were enrolled in this study. When this study was initiated, 90 patients provided written informed consent to measure the serum galectin-9 levels in the samples. One patient orally consented to this study, and the remaining 10 patients were dead and had no relatives listed in their medical records; therefore, the opt-out methods were applied.

The gender distribution of patients comprised 59 males and 42 females (Fig. 1). Viral infection was detected in 58 patients, of which 45 were positive for HCV and 13 positive for HBV. Nonviral cases included 19 patients with non-alcoholic fatty liver disease and 12 patients with primary biliary cholangitis, and the etiology of five cases was unknown. Diabetes mellitus was comorbid in 24 patients. CH was seen in 50 patients, and LC was diagnosed in 51 patients. Among cirrhotic cases, 35 patients were designated Child–Pugh class A and 16 cases were designated class B. HCC was noted in 46 cases, among which 45 were classified as either T1N0M0 or T2N0M0, and the last case was staged as T3aN0M0 according to UICC TNM classification system, 7th edition. Other clinical parameters including age, laboratory tests and indexes of liver fibrosis are presented in Table 1. The median value of the serum galectin-9 concentration was 77.54 pg/mL (interquartile range: 18.89–241.9 pg/mL) in total 101 cases, 108.90 pg/mL (59.57–277.5 pg/mL) in HCV-infected cases, 45.31 pg/mL (4.695–313.6 pg/mL) in HBV-infected cases, 48.11 pg/mL (6.632–108.9 pg/mL) in patients without HCV or HBV infection (Table 1). The distribution of serum galectin-9 levels is plotted in Figure S1.

**Correlation analyses between galectin-9 and independent variables.** The correlation between galectin-9 and the continuous variables was calculated using Spearman’s rank correlation coefficient (Figs S2 and S3). Among the nine tested variables, Fib-4 index, T-Bil, PT-INR, ALT, APRI, Plt, and M2BPGi were significantly correlated with the serum galectin-9 levels. Three indicators for liver fibrosis, M2BPGi, Fib-4 index, and APRI showed the greatest correlation coefficients with the serum galectin-9 levels.

Difference of median galectin-9 values by six categorical variables was assessed using Mann–Whitney U-test (Fig. S4). HBV or HCV infection status, LC, and presence of HCC were significantly correlated to the measured serum galectin-9 values.

**Multiple linear regression analysis.** To clarify which factors were able to predict the serum galectin-9 levels, multiple linear regression analysis was performed using EZR with the Akaike information criterion. All six continuous variables (age, ALT, Fib-4 index, T-Bil, PT-INR, and eGFR) and four categorical variables (gender, HBV or HCV infection status, diabetes mellitus, and presence of HCC) were included in the analysis. Galectin-9 concentration, ALT, Fib-4 index, and T-Bil were log-transformed because their distribution was far from normality. Multiple linear regression analysis resulted in an optimized model consisting of Fib-4 index, T-Bil, and the intercept. Fib-4 index alone was the statistically significant variable to predict serum galectin-9 concentration among the 10 variables (Table 2). The adjusted $R^2$ value was calculated as 0.2289, and the $F$-test of the model
Fib-4 index 0.5485 0.2146 2.557

A linear regression analysis using Fib-4 index was conducted. Post-hoc power test. The post-hoc power test for the multiple linear regression analysis using Fib-4 index was conducted to calculate the odds ratio of higher serum galectin-9 levels for liver fibrosis. A multiple logistic regression analysis was performed to calculate the odds ratio of higher serum galectin-9 levels for liver fibrosis. The median serum galectin-9 concentration of the Fib-4 index > 3.25 group was significantly higher than those with Fib-4 index equal to or less than 3.25 (P < 0.0001, Fig. 2a). When the galectin-9 cut-off value was set to the median value of 101 cases, 77.54 pg/mL, the sensitivity was 66.0% and specificity was 66.7% in detection of Fib-4 index > 3.25. When the cut-off value was set to the two times as large as the median value, 155 pg/mL, the specificity increased to 85.4% and the sensitivity decreased to 43.4%. A similar analysis was performed using APRI instead of Fib-4 indexes. The serum galectin-9 concentration of the APRI > 1.5 group was significantly higher than that of the other group (P < 0.0001, Fig. 2c). When the galectin-9 cut-off value was set to 77.54 pg/mL, the sensitivity was 75.8% and specificity was 82.4% and the sensitivity decreased to 54.6% (Fig. 2d).

Receiver operating characteristic analysis. Total 101 cases were classified into two groups by Fib-4 index = 3.25. The median serum galectin-9 concentration of the Fib-4 index > 3.25 group was significantly higher than those with Fib-4 index equal to or less than 3.25 (P < 0.0001, Fig. 2a). The area under the curve measured 0.7237 (P < 0.0001, Fig. 2b). When the galectin-9 cut-off value was set to the median value of 101 cases, 77.54 pg/mL, the sensitivity was 66.0% and specificity was 66.7% in detection of Fib-4 index > 3.25. When the cut-off value was set to the two times as large as the median value, 155 pg/mL, the specificity increased to 85.4% and the sensitivity decreased to 43.4%.

produce a P value less than 0.005. The variance inflation factors for the 10 variables ranged at levels lower than 5 (Table S1).

A multiple linear regression analysis using APRI instead of Fib-4 index as a liver fibrosis index was also performed (Table 3), for Fib-4 index was reported to lack accuracy in patients older than 65 years. APRI was also log-transformed because its distribution was far from normality. According to the optimized model, APRI also acted as a significant predictor of the serum galectin-9 concentration. The variance inflation factors for the 10 variables were limited below 5 (Table S2).

Post-hoc power test. The post-hoc power test for the multiple linear regression analysis using Fib-4 index was conducted using Free Statistics Calculators under conditions that number of predictors = 3; the adjusted R-squared value = 0.2289; significance level = 0.05; and the sample size = 101. The observed statistical power yielded 0.9990 in the current analyses above. Similarly, the statistical power for the multiple linear regression analysis using APRI was 0.9995.

Odds ratio of higher serum galectin-9 levels for liver fibrosis. A multiple logistic regression analysis was performed to calculate the odds ratio of higher serum galectin-9 levels for liver fibrosis. The median serum galectin-9 concentration of the APRI > 1.5 group was significantly higher than that of the other group (P < 0.0001, Fig. 2c). When the galectin-9 cut-off value was set to 77.54 pg/mL, the sensitivity was 75.8% and specificity was 82.4% and the sensitivity decreased to 54.6% (Fig. 2d).

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concentrations for liver fibrosis progression. The cohort was divided into 50 patients with CH and 51 patients with LC. Fib-4 index, APRI, Plt, and M2BPGi were significantly different between the two groups (Fig. S5). The odds ratios were processed with four independent variables, age, gender, viral infection, and log-transformed serum galectin-9 levels. As a result, 10 pg/mL increase in the serum galectin-9 concentrations presented a statistically significant odds ratio of 3.90 for liver fibrosis progression from CH to LC (Table 4). The variance inflation factors for the variables were around 1 (Table S3).

Discussion

The current study revealed elevated serum galectin-9 levels were predicted by Fib-4 index and APRI, scores of liver fibrosis but neither ALT, a representative biomarker of liver inflammation, nor the presence of HCC. The sample size of this analysis was sufficient to perform multiple linear regression analyses, and

### Table 4 Multiple logistic regression analysis

| Independent variables | Odds ratio | Lower limits of 95% CI | Upper limits of 95% CI | P value |
|-----------------------|------------|------------------------|------------------------|---------|
| (Intercept)           | < 0.0001   | 0.006                  | 0.015                  | < 0.0001|
| Galectin-9            | 3.900      | 1.090                  | 14.00                  | 0.0367  |
| Virus                 | 21.60      | 4.980                  | 93.20                  | < 0.0001|
| Gender                | 3.090      | 0.817                  | 11.70                  | 0.0966  |
| Age                   | 1.080      | 1.010                  | 1.160                  | 0.0326  |

CI, confidence interval

$^aP$ value < 0.05
post-hoc power analysis showed power values far greater than 0.8. Furthermore, a significant odds ratio of 3.90 was observed for the ability of higher serum galectin-9 concentrations to predict liver fibrosis progression.

Chronic liver diseases involve three pathological features— inflammation, fibrosis, and cancer. Therefore, three predictors were presumed to be independent variables for the serum galectin-9 levels; thus, we hypothesized that three parameters (ALT, Fib-4 index or APRI, and HCC) could reflect the galectin-9 values. However, the optimized model lacked ALT or HCC. Fib-4 index and APRI alone were identified as significant variables. These results suggest among liver inflammation, fibrosis, and cancer, fibrosis was best reflected by the serum galectin-9 concentration.

Hepatocellular carcinoma was excluded as an independent variable of the serum galectin-9 concentration in these analyses although 46 patients with HCC presented significantly higher Fib-4 indexes than 55 cases without HCC in this study (Fig. 3a). HCC often develops during the third stage of CLDs and usually manifests in cirrhotic livers. To mention galectin-9’s contribution to HCC biology, HCC itself was reported to decrease its galectin-9 expression comparing with adjacent tissue. Thus, our data regarding patients with HCC should be interpreted as the serum galectin-9 concentration in HCC patients is dependent on fibrotic changes in the background liver tissues.

The equation for Fib-4 index contains ALT and age. Although Fib-4 index significantly correlated with ALT and age (Fig. 3b,c), variable inflation factor for the two parameters were not elevated more than 5 (Table S1). Therefore, the analysis using the both parameters simultaneously was deemed appropriate post-hoc. In addition, the data showing a significant correlation between serum galectin-9 levels and liver fibrosis were validated by another multiple linear regression analysis using APRI instead of Fib-4 index because the equation for the APRI did not include ALT or age.

Total bilirubin also remained with Fib-4 index and APRI as independent variables in the optimized model (Tables 2 and 3). In the current study, 83 of 101 patients presented T-Bil values within normal limits, equal to or less than 1.2 mg/dL. Only four patients were featured by jaundice. Whether hyperbilirubinemia affects serum galectin-9 levels remains to be assessed.

This study did not include patients with either acute hepatitis or fulminant hepatitis, in which the serum galectin-9 levels were greater than 300 pg/mL. The prominent elevation of serum galectin-9 levels in acute and fulminant hepatitis cases might be the result of excess inflammatory responses as well as tissue injury resulting in the release of intracellular galectin-9 into the extracellular environment. In the current study, patients with either CH or LC were enrolled. The patients’ median ALT value remained near the upper limit of the normal range, and a 75% percentile value was less than 300 pg/mL (Table 1). Patients with CH or LC should be complicate with milder inflammatory responses and liver tissue injuries, and their serum galectin-9 levels should be predominantly determined by the degree of liver fibrosis.

Main parts of liver fibrosis are executed by hepatic stellate cells (HSCs). Macrophages in peripheral blood and hepatic Kupffer cells regulate activities of HSCs. All of HSCs, macrophages, and Kupffer cells are confirmed to produce galectin-9. As generally understood, galectin-9 plays pivotal roles in tissue fibrosis. Similar to other members of galectin family, galectin-9 is thought to bridge cell surface and extracellular matrix. However, whether and how galectin-9 contributes to progression of liver fibrosis remains further unclear in comparison with galectin-1 and galectin-3. Moreover, the current study was not able to reveal which type of cells secret galectin-9 into sera. The mechanisms of galectin-9 secretion are also to be clarified, for galectin-9 lacks a signal sequence to be secreted via endoplasmic reticulum–Golgi apparatus pathway.

The primary limitation of this study was no biopsy specimens, or surgically resected tissue samples taken at the same time of serum preservation were available. Therefore, no information comparing the pathological findings underlining inflammation or fibrosis in liver and serum galectin-9 concentrations was obtained. For the second limitation, the cohort was heterogeneous in etiology. Serum galectin-9 concentrations were significantly higher in HCV-infected cases than HBV-infected patients or uninfected patients. Higher Fib-4 index, APRI, and M2BPGi as well as

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Figure 3  Correlation analysis between Fibrosis-4 (Fib-4) index and hepatocellular carcinoma (HCC), age, or alanine aminotransferase (ALT). (a) The median Fib-4 indexes differed between patients with and without HCC as determined by Mann–Whitney U-test ($P < 0.05$). The data are presented as median and interquartile range. (b,c) The correlation analyses showed a significant correlation between Fib-4 index and ALT (b) and age (c) based on Spearman’s rank correlation coefficient ($P < 0.05$).
lower Plt in HCV-infected patients compared with patients with other etiologies revealed HCV-infected patients in this cohort exhibited a greater extent of liver fibrosis progression than other patients (Fig. 5e–h). The extent to which the different etiologies contributed to the difference in serum galectin-9 levels remained unclear in the current work.

In conclusion, the serum galectin-9 levels were correlated to liver fibrosis as indicated by Fib-4 index and APRI but were not correlated to either liver inflammation represented by ALT or HCC complications. Galectin-9 levels in the sera might be a promising alternative biomarker for liver fibrosis. Furthermore, higher serum galectin-9 levels were identified as a predictor for liver fibrosis progression. The mechanisms underlying the contribution of galectin-9 to liver fibrosis progression remain to be investigated. Comparison analyses between serum galectin-9 levels and other non-invasive biomarkers for detecting and quantifying liver fibrosis using liver pathological samples will be conducted.

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Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article.

Table S1. Variance inflation factors in multiple linear regression analysis including FIB-4 index.
Table S2. Variance inflation factors in multiple linear regression analysis including APRI.

Table S3. Variance inflation factors in the multiple logistic regression analysis.

Figure S1. Distribution of the serum galectin-9 levels as determined by ELISA.

Figures S2 and S3. Correlation analyses between the serum galectin-9 levels and the continuous parameters as determined using Spearman’s rank correlation coefficient \( P < 0.05 \). Among the 9 variables, ALT, Fib-4 index, T-Bil, PT-INR, APRI, platelet count and M2BPGi were significantly correlated with the serum galectin-9 levels. Three indicators for liver fibrosis, M2BPGi, Fib-4 index and APRI, showed the strongest correlation with the serum galectin-9 levels.

Figure S4. Correlation analyses between the serum galectin-9 levels and the categorical parameters as determined by Mann–Whitney U test \( P < 0.05 \). HBV or HCV infection status, liver cirrhosis and the presence of HCC were significantly correlated with the measured serum galectin-9 values. However, clinical staging of HCC did not significantly correlate with the galectin-9 levels. The data are presented as median and inter quartile range. DM, diabetes mellitus; CH, chronic hepatitis; LC, liver cirrhosis.

Figure S5. Four indicators of liver fibrosis, Fib-4 index, APRI, platelet count and M2BPGi in patients at each fibrotic stage and with each etiology. (a-d) The cohort of 101 patients was divided into 50 chronic hepatitis (CH) and 51 liver cirrhosis (LC) based on morphological changes in the liver figured by computed tomography or ultrasound sonography examinations and extrahepatic complications including esophageal or gastric varix and ascites. Fib-4 index, APRI, Plt and M2BPGi were significantly different between patients with CH and LC. (e-h) Fib-4 index, APRI, Plt and M2BPGi were determined with each etiology. HCV-infected 45 patients presented the greatest extent of liver fibrosis progression and non B non C 43 patients showed the least progression. The data are presented as median and inter quartile range. NBnC, non B and non C hepatitis virus infection.