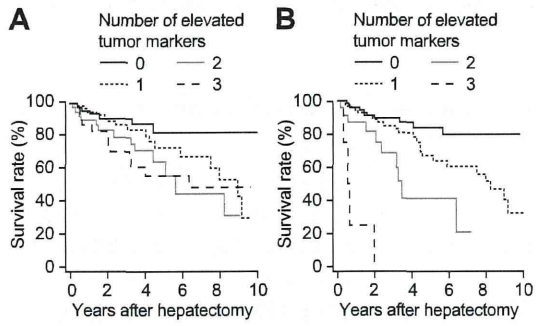


Fig. 1. Survival rates after hepatectomy as a function of the number of elevated tumor markers measured before and after hepatectomy. (A) Survival rates according to the number of elevated tumor markers measured before hepatectomy (zero *vs.* one, $p = 0.0907$; one *vs.* two, $p = 0.1542$; two *vs.* three, $p = 0.8772$). (B) Survival rates according to the number of elevated tumor markers measured after hepatectomy (zero *vs.* one, $p = 0.0322$; one *vs.* two, $p = 0.0085$; two *vs.* three, $p = 0.0006$).

with triple positive tumor markers have significantly lower recurrence-free and disease-specific survival rates after hepatectomy. Their study has included patients who underwent non-anatomical hepatectomy (35.7%). This treatment with insufficient curativity might have increased the impact of triple positive pre-treatment tumor markers and associated progression of HCC on the prognosis of patients after hepatectomy. In contrast to their results, we did not find any difference in survival and recurrence rates between patients with triple positive tumor markers and other patients in the pretreatment evaluation, for patients who underwent anatomical hepatectomy. In addition, they failed to find any differences in the rates between patients with one or two positive tumor markers and those without positive tumor markers, measured before treatment. With respect to the post-treatment evaluation in our study, decreased survival rates and increased recurrence rates were observed, not only in patients with elevation of all three tumor markers but also in patients with one or two elevated tumor markers, when compared to those with no elevated tumor markers. Thus, the number of elevated tumor markers after treatment was well associated with prognosis after curative hepatectomy and categorized patients into 4 groups by the likelihood of survival and recurrence.

An increase in tumor size and the rate of portal vein invasion, and a decrease in the rate of well-differentiated HCC and of HCC

Table 3. Univariate and multivariate analyses for factors associated with postoperative survival using a combination of three tumor markers measured after hepatectomy (n = 173).

	Univariate analyses		Multivariate analyses	
Factor	*p* value	Risk ratio (95% CI)	*p* value	Risk ratio (95% CI)
Age	0.0518	1.0358 (0.9997-1.0766)	0.1032	1.0320 (0.9939-1.0753)
Sex				
Male		1		
Female	0.3682	0.8371 (0.5350-1.2138)		
Child-Pugh class				
A		1		
B	0.2379	0.6038 (0.6039-1.2951)		
Tumor size	0.0012	1.1625 (1.0671-1.2497)	0.2049	1.0639 (0.9650-1.1651)
Number of tumors	0.0006	2.2953 (1.4681-3.4226)	0.0370	1.7047 (1.0345-2.7230)
Differentiation				
Well-		1		
Moderately/poorly	0.0009	1.7532 (1.2453-2.6068)	0.0554	1.4437 (0.9918-2.1943)
Growth pattern				
Expansive		1		
Infiltrative	0.0142	1.6225 (1.1114-2.2602)	0.3353	1.2306 (0.7984-1.8370)
Macroscopic portal vein invasion				
Absent		1		
Present	0.2168	1.5151 (0.7443-2.5195)		
Microscopic portal vein invasion				
Absent		1		
Present	0.0083	1.5829 (1.1327-2.1604)	0.5730	1.1162 (0.7554-1.6191)
Number of positive tumor markers				
0		1		
1	0.0315	1.4807 (1.0344-2.1980)	0.0194	1.5534 (1.0720-2.3312)
2	0.0004	2.3463 (1.4928-3.6906)	0.0172	1.8241 (1.1157-2.9683)
3	<0.0001	6.0824 (3.0998-10.9708)	0.0018	3.6788 (1.7020-7.3886)

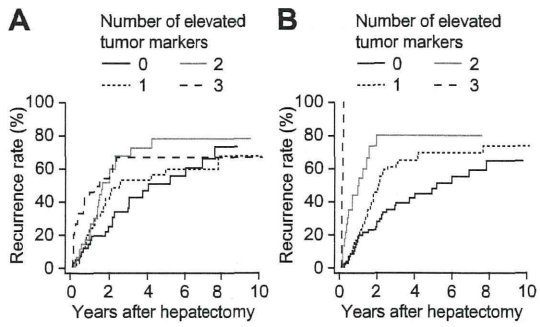


Fig. 2. Recurrence rates after hepatectomy as a function of the number of elevated tumor markers measured before and after hepatectomy. (A) Recurrence rates according to the number of elevated tumor markers measured before hepatectomy (zero *vs.* one, $p = 0.4966$; one *vs.* two, $p = 0.1756$; two *vs.* three, $p = 0.6227$). (B) Recurrence rates according to the number of elevated tumor markers measured after hepatectomy (zero *vs.* one, $p = 0.0352$; one *vs.* two, $p = 0.0050$; two *vs.* three, $p < 0.0001$).

with expansive growth were found in association with the number of elevated tumor markers before treatment, as in our previous report [8] and the report by Kiriyama *et al.* [22]. These associations lacked with the number of elevated tumor markers after treatment. Although pretreatment elevations of HCC tumor markers, especially AFP-L3 and DCP, have been reported to indicate advanced characteristics of HCC with poor prognosis [23,24], the effects markedly decreased when patients underwent hepatectomy [25]. Hepatectomy appears to effectively treat HCC with high malignant potential associated with a pretreatment elevation of tumor markers, if hepatectomy is performed anatomically with curative intent. Indeed, the number of elevated tumor markers markedly decreased after treatment in patients who underwent hepatectomy compared to patients treated with locoregional ablative therapies (radiofrequency ablation or ethanol injection) or transcatheter arterial chemoembolization [8]. Therefore, the elevation of tumor markers after hepatectomy may indicate the residual minute HCC cells that cannot be identified during hepatectomy and imaging examination after treatment. Patients in whom all three tumor markers remained positive even after hepatectomy had markedly low survival and high recurrence

Table 4. Univariate and multivariate analyses for factors associated with postoperative recurrence using a combination of three tumor markers measured after hepatectomy (n = 173).

	Univariate analyses		Multivariate analyses	
Factor	*p* value	Risk ratio (95% CI)	*p* value	Risk ratio (95% CI)
Age	0.1167	1.0185 (0.9957-1.0437)		
Sex				
Male		1		
Female	0.5384	0.9220 (0.6971-1.1841)		
Child-Pugh class				
A		1		
B	0.7533	1.0701 (0.6675-1.5515)		
Tumor size	<0.0001	1.1745 (1.0994-1.2431)	0.1180	1.0764 (0.9815-1.1826)
Number of tumors	0.0023	1.8096 (1.2539-2.5105)	0.0329	1.5975 (1.0409-2.3558)
Differentiation				
Well-		1		
Moderately/poorly	0.0222	1.2817 (1.0354-1.6045)	0.2196	1.1620 (0.9149-1.4852)
Growth pattern				
Expansive		1		
Infiltrative	0.2978	1.1830 (0.8512-1.5707)		
Macroscopic portal vein invasion				
Absent		1		
Present	0.0123	1.8793 (1.1690-2.7380)	0.1300	1.6593 (0.8519-2.9452)
Microscopic portal vein invasion				
Absent		1		
Present	0.0504	1.3051 (0.9995-1.6620)	0.4978	1.1115 (0.8121-1.4897)
Number of positive tumor markers				
0		1		
1	0.0413	1.2716 (1.0095-1.6088)	0.0069	1.4017 (1.0967-1.8044)
2	0.0001	1.9214 (1.3999-2.5862)	0.0001	2.0143 (1.4590-2.7324)
3	<0.0001	11.8230 (5.5814-25.0773)	<0.0001	8.3969 (3.4707-19.7694)

rates; elevation of all three tumor markers after treatment was a strongest indicator of poor survival. These patients should be considered to have received insufficient resection despite anatomical hepatectomy with curative intent and the absence of residual HCC tumors on CT examination after hepatectomy.

The recurrence rates were high regardless of the number of post-treatment tumor markers; recurrence was detected in more than 40% of patients, even in patients without elevated tumor markers after hepatectomy (Fig. 2). This is partly due to the high rate of recurrence of HCC even after curative treatment including multicentric occurrence [26]. Therefore, the sensitivity of the elevated post-treatment tumor markers in predicting recurrence is not high and, conversely, the absence of elevated tumor markers after treatment does not necessarily indicate a low risk of recurrence. However, the time to recurrence after treatment is an important factor for prognosis, and the number of elevated tumor markers after hepatectomy well discriminated recurrence rates when the time to recurrence was taken into account.

This study has several limitations. All three post-treatment tumor markers were measured at one time in the same serum sample despite differences in the half-lives between AFP/AFP-L3 and DCP, and was not strictly based on the half-lives of each marker. It was difficult to obtain serum samples at multiple occasions in a short period, after hepatectomy in clinical settings. We, therefore, measured all tumor markers between 1 and 2 months after hepatectomy, considering that the values of post-treatment tumor markers were not influenced by the pretreatment elevation during this period. Since none of our patients were treated with liver transplantation, we do not have data on the changes in the number of elevated tumor markers when patients undergo liver transplantation. Such analysis should be performed in the future. In addition, the association between number of elevated post-treatment tumor markers and status of the remnant liver after hepatectomy, including the residue of minute HCC cells undetected by imaging modalities, remains unknown. However, we hope this will be investigated in the future to shed light on the mechanisms behind the elevation of tumor markers after hepatectomy.

In conclusion, in our examination of 173 patients treated with hepatectomy with curative intent, the combination of three tumor markers measured after treatment had high discriminatory ability for survival and recurrence after hepatectomy. Further studies are warranted to confirm this association in other populations.

Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jhep.2012.07.018.

References

[1] Parkin D, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin 2002;55:74–108.

[2] Befeler AS, DiBisceglie AM. Hepatocellular carcinoma: diagnosis and treatment. Gastroenterology 2002;122:1609–1619.

[3] Umemura T, Kiyosawa K. Epidemiology of hepatocellular carcinoma in Japan. Hepatol Res 2007;37:S95–100.

[4] Tsukuma H, Hiyama T, Tanaka S, Nakao M, Yabuuchi T, Kitamura T, et al. Risk factors for hepatocellular carcinoma among patients with chronic liver disease. N Engl J Med 1993;328:1797–1801.

[5] Taketa K, Sekiya C, Namiki N, Akamatsu K, Ohta Y, Endo Y, et al. Lectin-reactive profiles of alpha-fetoprotein characterizing hepatocellular carcinoma and related conditions. Gastroenterology 1990;99:508–518.

[6] Liebman HA, Furie BC, Tong MJ, Blanchard RA, Lo KJ, Lee SD, et al. Des-gamma-carboxy (abnormal) prothrombin as a serum marker of primary hepatocellular carcinoma. N Engl J Med 1984;310:1427–1431.

[7] Fujiyama S, Tanaka M, Maeda S, Ashihara H, Hirata R, Tomita K. Tumor markers in early diagnosis, follow-up and management of patients with hepatocellular carcinoma. Oncology 2002;62:S57–S63.

[8] Toyoda H, Kumada T, Kiriyama S, Sone Y, Tanikawa M, Hisanaga Y, et al. Prognostic significance of simultaneous measurement of three tumor markers in patients with hepatocellular carcinoma. Clin Gastroenterol Hepatol 2006;4:111–117.

[9] Kang SH, Kim DY, Jeon SM, Ahn SH, Park JY, Kim SU, et al. Clinical characteristics and prognosis of hepatocellular carcinoma with different sets of serum AFP and PIVKA-II levels. Eur J Gastroenterol Hepatol 2012;24:849–856.

[10] Kokudo N, Makuuchi M. Evidence-based clinical practice guidelines for hepatocellular carcinoma in Japan: J-HCC guidelines. J Gastroenterol 2009;44:S119–121.

[11] Ramerz R. AFP (alpha-fetoprotein). In: Thomas L, editor. Clinical and Laboratory Diagnosis. Frankfurt/Main: TH-Books Verlagsgesellschaft mbH; 1998. p. 941–945.

[12] Furukawa M, Nakanishi T, Okuda H, Ishida S, Obata H. Changes of plasma des-γ-carboxy prothrombin levels in patients with hepatocellular carcinoma in response to vitamin K. Cancer 1992;69:31–38.

[13] Kagebayashi C, Yamaguchi I, Akinaga A, Kitano H, Yokoyama K, Satomura M, et al. Automated immunoassay system for AFP-L3% using on-chip electrokinetic reaction and separation by affinity electrophoresis. Anal Biochem 2009;388:306–311.

[14] Oka H, Tamori A, Kuroki T, Kobayashi K, Yamamoto S. Prospective study of alpha-fetoprotein in cirrhotic patients monitored for development of hepatocellular carcinoma. Hepatology 1994;19:61–66.

[15] Koda M, Murawaki Y, Mitsuda A, Ohyama K, Horie Y, Suou T, et al. Predictive factors for intrahepatic recurrence after percutaneous ethanol injection therapy for small hepatocellular carcinoma. Cancer 2000;88:529–537.

[16] Toyoda H, Kumada T, Tada T, Kaneoka Y, Maeda A, Kanke F, et al. Clinical utility of high sensitive *Lens culinaris* agglutinin-reactive alpha-fetoprotein in hepatocellular carcinoma patients with alpha-fetoprotein <20 ng/ml. Cancer Sci 2011;102:1025–1031.

[17] Okuda H, Nakanishi T, Takatsu K, Saito A, Hayashi N, Takasaki K, et al. Serum levels of des-gamma-carboxy prothrombin measured using the revised enzyme immunoassay kit with increased sensitivity in relation to clinicopathological features of solitary hepatocellular carcinoma. Cancer 2000;88:544–549.

[18] Kaplan EL, Meier P. Non parametric estimation for incomplete observation. J Am Stat Assoc 1958;53:457–481.

[19] Petro R, Pike MC. Conservation of the approximation (0–E2)/E in the log rank test for survival data on tumor incidence data. Biometrics 1973;29:579–584.

[20] Cox D. Regression models and life tables. J R Stat Soc 1972;34:187–220.

[21] Pugh RNH, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R. Transection of the oesophagus for bleeding oesophageal varices. Br J Surg 1973;60:646–649.

[22] Kiriyama S, Uchiyama K, Ueno M, Ozawa S, Hayami S, Tani M, et al. Triple positive tumor markers for hepatocellular carcinoma are useful predictor of a poor survival. Ann Surg 2011;254:984–991.

[23] Aoyagi Y, Isokawa O, Suda T, Watanabe M, Suzuki Y, Asakura H. The fucosylation index of alpha-fetoprotein as a possible prognostic indicator for patients with hepatocellular carcinoma. Cancer 1998;83:2076–2082.

[24] Koike Y, Shiratori Y, Sato S, Obi S, Teratani T, Imamura M, et al. Des-gamma-carboxy prothrombin as a useful predisposing factor for the development of portal venous invasion in patients with hepatocellular carcinoma: a prospective analysis of 227 patients. Cancer 2001;91:561–569.

[25] Toyoda H, Kumada T, Kaneoka Y, Maeda A. Amino acid substitutions in the hepatitis C virus core region are associated with postoperative recurrence and survival of patients with HCV genotype 1b-associated hepatocellular carcinoma. Ann Surg 2011;254:326–332.

[26] Kumada T, Nakano S, Takeda I, Sugiyama K, Osada T, Kiriyama S, et al. Patterns of recurrence after initial treatment in patients with small hepatocellular carcinoma. Hepatology 1997;25:87–92.

Clinica Chimica Acta 413 (2012) 1796–1799

Contents lists available at SciVerse ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

Comparison of LecT-Hepa and FibroScan for assessment of liver fibrosis in hepatitis B virus infected patients with different ALT levels

Dongning Du [a,b,c,1], Xuejuan Zhu [a,1], Atsushi Kuno [b], Atsushi Matsuda [b], Chikayuki Tsuruno [d], Demin Yu [a], Yan Zhang [c,e], Yuzuru Ikehara [b], Yasuhito Tanaka [f], Xinxin Zhang [a,*], Hisashi Narimatsu [b,c,**]

[a] Department of Infectious Diseases, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, 197, Ruijin Er Road, Shanghai 200025, China
[b] Research Center for Medical Glycoscience (RCMG), National Institute of Advanced Industrial Science and Technology (AIST), 1-1-1 Umezono, Tsukuba, Ibaraki 305-8568, Japan
[c] SCSB (China) - AIST (Japan) Joint Medical Glycomics Laboratory, 800 Dong Chuan Road, Minhang, Shanghai 200240, China
[d] Product Development Div. 2, Sysmex Corporation, 4-4-4 Takatsukadai, Nishi-ku, Kobe, Hyogo 651-2271, Japan
[e] Ministry of Education Key Laboratory of Systems Biomedicine, Shanghai Center for Systems Biomedicine (SCSB), Shanghai Jiao Tong University, 800 Dong Chuan Road, Minhang, Shanghai 200240, China
[f] Department of Virology and Liver Unit, Nagoya City University Graduate School of Medical Sciences, Kawasumi, Mizuho, Nagoya, Aichi 467-8601, Japan

ARTICLE INFO

Article history:
Received 12 June 2012
Received in revised form 5 July 2012
Accepted 5 July 2012
Available online 13 July 2012

Keywords:
Liver fibrosis
Glycomarker
Hepatitis B
Cirrhosis
Alanine aminotransferase

ABSTRACT

Background: FibroScan is one of the noninvasive techniques based on the transient elastography that can assess the progression of liver fibrosis in chronic hepatitis patients in daily clinical practice. Recently, LecT-Hepa was validated as a serological glycomarker correlating well with the fibrosis stage determined by liver biopsy, and was superior to many other noninvasive biochemical markers and tests. We compared the reliability of LecT-Hepa with that of FibroScan for evaluation of liver fibrosis.
Methods: The effects of increased alanine aminotransferase (ALT) activities on LecT-Hepa and FibroScan were investigated.
Results: The areas under the receiver-operating characteristic curves, sensitivity and specificity for detecting cirrhosis, which is one of the outcomes of fibrosis estimation, were 0.82, 72.5% and 78.2% of LecT-Hepa, 0.85, 87.0% and 74.1% of FibroScan; these did not differ significantly. The count distribution of LecT-Hepa in non-cirrhosis group or cirrhosis group did not differ between the patients grouped according to their ALT levels, whereas that of FibroScan was substantially affected.
Conclusion: LecT-Hepa was confirmed as a reliable noninvasive test for the evaluation of liver fibrosis in hepatitis B virus-infected patients with comparable performance to that of FibroScan and proved to be unaffected by inflammation.

1. Introduction

It is estimated that about 2 billion people worldwide have been infected with the hepatitis B virus (HBV), and >350 million of them have chronic HBV infection [1]. In China, a seroepidemiological survey of HBV infection in 2006 showed that the prevalence of hepatitis B surface antigen positivity was 7.18%. It was estimated that 93 million people were HBV carriers, of whom 30 million were patients with chronic hepatitis B (CHB) [2]. CHB may progress to cirrhosis and hepatocellular carcinoma. An accurate method for monitoring the progression of liver fibrosis is urgently needed for the prognosis and management of chronic liver diseases. Liver biopsy is generally considered as the gold standard for assessing hepatic histology in CHB [3–5]. However, it often has limitations due to its invasiveness, risk of complications, sampling errors, and interobserver variability [6–8]. Many noninvasive methods for replacing or complementing the liver biopsy have been developed in recent years [9–12]. FibroScan (transient elastography) and FibroTest (serological marker test) have been evaluated most frequently; these methods have similar diagnostic accuracies for predicting fibrosis staging from receiver-operating characteristic (ROC) curves [13–16]. FibroTest employs a narrow and complex algorithm for 5 biochemical markers (α2-macroglobulin, apolipoprotein A1, haptoglobin, γ-glutamyl transferase, and bilirubin), which requires extensive and specialized blood analysis [17]. Recently, we developed a novel diagnostic score named LecT-Hepa for convenient and rapid monitoring of liver fibrosis progression. It is based on glyco-alteration (e.g., fucosylation and desialylation) of serum α1-acid glycoprotein

Abbreviations: HBV, hepatitis B virus; CHB, chronic hepatitis B; PLT, platelet count; AGP, α1-acid glycoprotein; LSM, liver stiffness measurement; LC, liver cirrhosis; non-LC, non-cirrhosis; DSA, *Datura stramonium* agglutinin; MAL, *Maackia amurensis* lectin; AOL, *Aspergillus oryzae* lectin.

* Corresponding author.
** Correspondence to: H. Narimatsu, Research Center for Medical Glycoscience (RCMG), National Institute of Advanced Industrial Science and Technology (AIST), 1-1-1 Umezono, Tsukuba, Ibaraki 305-8568, Japan.
E-mail addresses: zxx10375@rjh.com.cn (X. Zhang), h.narimatsu@aist.go.jp (H. Narimatsu).
[1] These authors contributed equally to this paper.

0009-8981/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.cca.2012.07.005

(AGP), which is assessed using a triplex lectin–antibody immunoassay [18,19]. It has been demonstrated to be well correlated with the fibrosis stage determined by liver biopsy, and verified to be more efficient by comparing with other serological methods (hyaluronic acid, tissue inhibitor of metalloproteases-1, platelet count, APRI, Forns index, Fib-4 index, and Zeng's score) in a multicenter study [20]. Here, to evaluate the reliability of LecT-Hepa for assessing liver fibrosis, we compared the diagnostic performance of LecT-Hepa and FibroScan for distinguishing cirrhosis from non-cirrhosis in a large cohort of HBV-infected Chinese patients with different serum alanine aminotransferase (ALT) levels.

2. Materials and methods

2.1. Patients

A total of 239 patients who had been positive for hepatitis B surface antigen for at least 6 months were enrolled retrospectively from Ruijin Hospital (Shanghai, China) from March 2009 to May 2011. Patients who were coinfected with another hepatitis virus or HIV, or who had excessive alcohol intake (>20 g/d), hepatocellular carcinoma, or other causes of liver diseases were excluded. For all patients, serum biochemical parameters, including the levels of aspartate aminotransferase (AST) and ALT, as well as platelet (PLT), were assessed at the time of the liver stiffness measurement. Normal values for ALT and AST ranged between 10 and 64 IU/l and between 8 and 40 IU/l, respectively, which were determined based on the manufacturer's instructions and adjusted according to the results of validation test by medical laboratory of Ruijin Hospital. Serum samples were collected at the time of the liver stiffness measurement for detection of lectins and stored at −20 °C until analysis. The patients were divided into two groups: liver cirrhosis (LC) group and non-cirrhosis (non-LC) group. The diagnosis of cirrhosis was based on clinical and morphological criteria and ultrasonography according to standard definitions [21]. The institutional ethics committees of Ruijin Hospital of Shanghai Jiao Tong University approved this study, and the informed consent was obtained from all patients.

2.2. Liver stiffness measurement

Liver stiffness was measured by transient elastography using FibroScan (EchoSens, Paris, France). The measurement depth was between 25 mm and 65 mm. For each patient, 10 validated measurements were performed. The success rate was calculated as the number of validated measurements divided by the total number of measurements. The results were expressed in kilopascals. The median value was considered representative of the elastic modulus of the liver. Only procedures with 10 validated measurements and a success rate of at least 60% were considered reliable.

2.3. Automatic acquisition of quantitative glyco-alteration of AGP

Each individual serum (5 μl) that had been stored at −20 °C was diluted 10 fold with phosphate buffered saline containing 0.2% sodium dodecyl sulfate, and then heated at 95 °C for 20 min. AGP in the resulting solution was enriched by immunoprecipitation with biotinylated anti-AGP antibody using an automated protein purification system (ED-01; GP BioSciences Ltd., Tokyo, Japan). Each elution fraction (100 μl) was kept at −80 °C until a sandwich immunoassay was performed. Subsequent to the enrichment, fibrosis-specific glyco-alteration of AGP was quantified using simultaneous lectin–antibody sandwich immunoassays for three lectins: *Datura stramonium* agglutinin (DSA), *Maackia amurensis* lectin (MAL), and *Aspergillus oryzae* lectin (AOL), by a fully automatic chemiluminescence enzyme immunoassay system (HISCL-2000i; Sysmex Co., Kobe, Japan). The criterion formula of LecT-Hepa was as before described [19]:

$$\text{LecT-Hepa} = \text{Log}_{10}[\text{AOL/DSA}] \times 8.6 - [\text{MAL/DSA}].$$

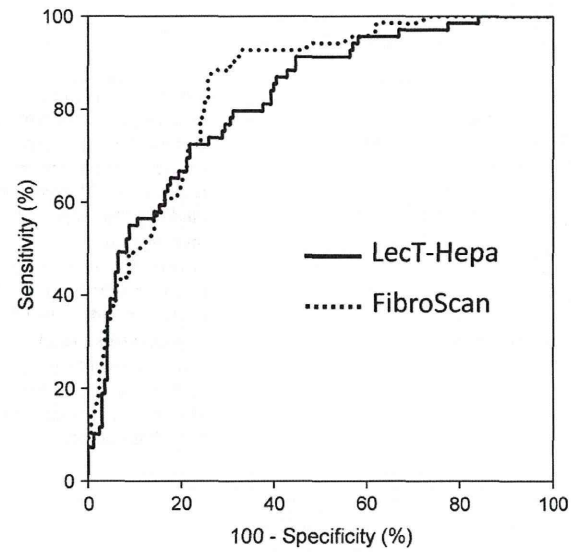
2.4. Statistical analysis

Statistical calculations were performed using software from GraphPad Prism 5 (GraphPad, San Diego, CA). A P value of <0.01 (1%) was considered to be statistically significant. The diagnostic performance of the fibrosis markers and indices were assessed using ROC curves and were then expressed as diagnostic specificity, sensitivity,

Table 1
Clinical characteristics of the patients.

Data	non-LC ($n=170$)	LC ($n=69$)	Significance non-LC vs LC
Age (y)	38.5±11.0	47.94±9.0	$P<0.0001$
Male sex (%)	126 (74.1%)	51 (73.9%)	–
AST (IU/l)	70.5±150.1	88.4±109.8	$P=0.0002$
ALT (IU/l)	111.6±213.7	88.5±116.1	$P=0.1965$
PLT ($\times 10^9$/l)	167.5±43.9	86.0±48.0	$P<0.0001$
FibroScan	10.3±8.8	27.0±19.1	$P<0.0001$
MAL/DSA	10.1±2.0	7.5±2.3	$P<0.0001$
AOL/DSA	5.1±13.5	24.0±47.6	$P<0.0001$

Patients were classified as non-LC or LC. LC, liver cirrhosis; non-LC, non-cirrhosis. Quantitative results are expressed as means±standard deviations or n (%).



	FibroScan	LecT-Hepa
AUC	0.85	0.82
(95% CI)	(0.797-0.897)	(0.763-0.877)
Se (%)	87.0	72.5
Sp (%)	74.1	78.2
PPV (%)	57.7	57.5
NPV (%)	93.3	87.5

Fig. 1. Receiver-operating characteristic curves of LecT-Hepa and FibroScan for distinguishing LC from non-LC. AUC, area under the receiver-operating characteristic curve; Se, sensitivity; Sp, specificity; PPV, positive predictive value; NPV, negative predictive value.

positive predictive value (PPV), negative predictive value (NPV) and area under the ROC curve (AUC) values (95% confidence interval [95% CI]).

3. Results

3.1. General characteristics

A total of 239 patients who showed evidence of chronic HBV infection and had undergone liver stiffness measurement were investigated. The mean age was 41.2 ± 11.3 y, and 177 (74%) of them were males. Among the all, 170 (71%) and 69 (29%) patients were diagnosed as non-LC and LC, respectively. Their characteristics are summarized in Table 1. Significant differences were found in Age ($P<0.0001$), AST ($P=0.0002$), PLT ($P<0.0001$), FiborScan ($P<0.0001$), MAL/DSA ($P<0.0001$), and AOL/DSA ($P<0.0001$) between the non-LC group and LC group, whereas ALT ($P=0.1965$) was not significantly different between the two groups.

3.2. Receiver-operating characteristic analysis

The overall diagnosis performances of LecT-Hepa and FibroScan were assessed using ROC curves. Fig. 1 shows the ROC curves for distinguishing LC from non-LC by both methods. The area under the ROC curve (95% CI) was 0.82 (0.763–0.877) for LecT-Hepa and 0.85 (0.797–0.897) for FibroScan. The overall diagnostic accuracies for LecT-Hepa and FibroScan were 77% and 78%, respectively. The obtained values for sensitivity, specificity, PPV, and NPV are shown in the bottom table of Fig. 1. There was no significant difference between both methods.

3.3. Effect of hepatic inflammation on the diagnostic cutoff values

Because the upper limit of the normal value for ALT level was 64 IU/l, the patients were categorized by the normal (≤64 IU/l) and elevated (>64 IU/l) ALT levels. According to this classification, 169 patients (71%) had the normal ALT level and 70 patients (29%) had the elevated ALT level. The proportions of patients with LC in the normal and elevated ALT levels were similar (28% of normal ALT patients and 30% of elevated ALT patients). Distribution of the values obtained by each test is shown in Fig. 2. Medians of these methods increased significantly between the non-LC group and LC group (all $P<0.0001$) in the both ALT levels. LecT-Hepa values in the non-LC group ($P=0.65$) and LC group ($P=0.02$) showed no significant difference between the two ALT categories (Fig. 2A). In contrast, the FibroScan value was obviously increased with the elevation of ALT levels ($P<0.0001$) even in the same diagnostic group (Fig. 2B). Thereby, we could distinguish the LC group in the normal ALT level from non-LC group in the elevated ALT level ($P<0.0001$) by LecT-Hepa, but could not by FibroScan ($P=0.05$). Collectively, the value of FibroScan was greatly affected by the ALT levels, whereas the value for LecT-Hepa was not influenced regardless of the ALT levels.

4. Discussion

This is the first study comparing LecT-Hepa with FibroScan. These results showed the obvious advantage of LecT-Hepa in comparison with FibroScan based on robustness against fluctuation of the ALT levels with a large cohort of HBV-infected Chinese patients at different ALT levels. Thus, the diagnostic performance of LecT-Hepa was the most reliable for monitoring the progression of hepatic fibrosis.

A recent paper showed that the majority of nucleoside-naïve patients with CHB who were treated with entecavir in the long-term cohort achieved substantial histological improvement and regression of fibrosis or cirrhosis [22], suggesting that a noninvasive test for the assessment of liver fibrosis in the treated patients is required during the follow-up. The liver biopsy is limited not only by its invasive nature, but also by its accuracy. A specimen collected in a standard liver biopsy using a short, narrow-gauge needle represents a very small portion of the whole liver mass, resulting in intra- and interobserver variability and sampling errors, which account for 25% of false-negative diagnoses of cirrhosis [23–25]. Therefore, a noninvasive marker that accurately reflects the condition of the whole liver is required.

At present, FibroScan is the most intensively evaluated noninvasive method for the assessment of liver fibrosis. Its diagnostic value is considered to be superior to that of biochemical markers [26]. However, several studies noted that liver stiffness measurements using FibroScan for patients with inflammation and acute liver damage overestimate the actual stage of fibrosis and may reduce the diagnostic accuracy [27,28]. In general, a high ALT level reflects a vigorous immune response

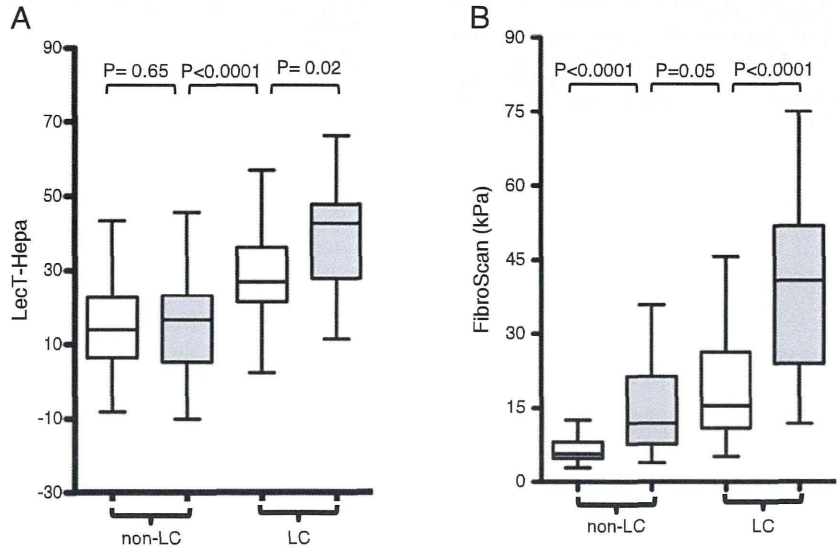


Fig. 2. Distribution of LecT-Hepa (A) and FibroScan (B) values in non-LC and LC patients with different ALT levels. The top and bottom of the whiskers are the 95th and 5th percentiles. The top and bottom of the boxes are the first and third quartiles. The size of the box represents the interquartile range within which 50% of the values are located. The line across the box indicates the median value. LC, liver cirrhosis; non-LC, non-cirrhosis. The open and gray boxes indicate normal (≤64 IU/l) and elevated (>64 IU/l) ALT levels, respectively.

to HBV and histological activity (i.e., necroinflammation). Our study obviously showed that the FibroScan values were substantially affected by ALT fluctuation. These results were also in accordance with the study of Kim et al., in which advanced fibrosis stage (F3–4) or cirrhosis showed a negative correlation with discordance between liver biopsy and FiborScan in assessing liver fibrosis in patients with CHB, and maximal activity grade 3–4 significantly influenced the liver stiffness measurement values in F3 and F4 [28]. In practice, hepatic activation and fibrosis stage should be estimated independently, as should histological diagnoses followed by a biopsy, such as the histological activity index scoring system. Thus, a marker that relies on an analysis of the specific protein content to monitor liver fibrosis should be robust against hepatic inflammation. In this context, we can explain that the reliability of LecT-Hepa is superior to that of FibroScan. LecT-Hepa has been already validated for estimating liver fibrosis using a large amount of serum specimens from patients with well-defined fibrosis stage by biopsy in a multicenter study [21]. This report led us to consider that LecT-Hepa can be a good substitute for liver biopsy. This is the reason we herein focused on the examination into the effect of hepatic inflammation on diagnosis of LC by LecT-Hepa.

In conclusion, we confirmed that LecT-Hepa is unaffected by inflammation. This suggested that LecT-Hepa is the most reliable and effective for the assessment of fibrosis progression in HBV-infected patients whose ALT levels are often fluctuated and thus can be used for routine assessments of liver fibrosis in HBV-infected patients.

Acknowledgments

The authors thank all those who helped with the measurements and the collection of serum samples, including K. Saito, S. Unno, T. Fukuda, M. Sogabe (AIST), W. Li, Y. Xu, and B. Tan (SCSB). The authors are also grateful to Y. Chiba (AIST), S. Nagai and Y. Takahama (Sysmex Co.) for critical discussion.

References

[1] Lavanchy D, Hepatitis B. virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. J Viral Hepat 2004;11: 97–107.
[2] Lu FM, Zhuang H. Management of hepatitis B in China. Chin Med J-Peking 2009;122: 3-4.
[3] Afdhal N, McHutchison J, Brown R, et al. Thrombocytopenia associated with chronic liver disease. J Hepatol 2008;48:1000-7.
[4] Alberti A, Clumeck N, Collins S, et al. Short statement of the first European Consensus Conference on the treatment of chronic hepatitis B and C in HIV co-infected patients. J Hepatol 2005;42:615-24.
[5] Hoofnagle JH, Doo E, Liang TJ, Fleischer R, Lok AS. Management of hepatitis B: summary of a clinical research workshop. Hepatology 2007;45:1056-75.
[6] Colloredo G, Guido M, Sonzogni A, Leandro G. Impact of liver biopsy size on histological evaluation of chronic viral hepatitis: the smaller the sample, the milder the disease. J Hepatol 2003;39:239-44.
[7] Bravo AA, Sheth SG, Chopra S. Liver biopsy. N Engl J Med 2001;344:495-500.
[8] Piccinino F, Sagnelli E, Pasquale G, Giusti G. Complications following percutaneous liver biopsy. A multicentre retrospective study on 68,276 biopsies. J Hepatol 1986;2:165-73.
[9] Castera L. Transient elastography and other noninvasive tests to assess hepatic fibrosis in patients with viral hepatitis. J Viral Hepat 2009;16:300-14.
[10] Forns X, Ampurdanes S, Llovet JM, et al. Identification of chronic hepatitis C patients without hepatic fibrosis by a simple predictive model. Hepatology 2002;36:986-92.
[11] Patel K, Gordon SC, Jacobson I, et al. Evaluation of a panel of non-invasive serum markers to differentiate mild from moderate-to-advanced liver fibrosis in chronic hepatitis C patients. J Hepatol 2004;41:935-42.
[12] Rosenberg WM, Voelker M, Thiel R, et al. Serum markers detect the presence of liver fibrosis: a cohort study. Gastroenterology 2004;127:1704-13.
[13] Sandrin L, Fourquet B, Hasquenoph JM, et al. Transient elastography: a new noninvasive method for assessment of hepatic fibrosis. Ultrasound Med Biol 2003;29: 1705-13.
[14] Castera L, Vergniol J, Foucher J, et al. Prospective comparison of transient elastography, fibrotest, APRI, and liver biopsy for the assessment of fibrosis in chronic hepatitis C. Gastroenterology 2005;128:343-50.
[15] Friedrich-Rust M, Ong MF, Martens S, et al. Performance of transient elastography for the staging of liver fibrosis: a meta-analysis. Gastroenterology 2008;134: 960-74.
[16] Shaheen AA, Wan AF, Myers RP. FibroTest and FibroScan for the prediction of hepatitis C-related fibrosis: a systematic review of diagnostic test accuracy. Am J Gastroenterol 2007;102:2589-600.
[17] Imbert-Bismut F, Ratziu V, Pieroni L, Charlotte F, Benhamou Y, Poynard T. Biochemical markers of liver fibrosis in patients with hepatitis C virus infection: a prospective study. Lancet 2001;357:1069-75.
[18] Turner GA. N-glycosylation of serum proteins in disease and its investigation using lectins. Clin Chim Acta 1992;208:149-71.
[19] Kuno A, Ikehara Y, Tanaka Y, et al. LecT-Hepa: a triplex lectin-antibody sandwich immunoassay for estimating the progression dynamics of liver fibrosis assisted by a bedside clinical chemistry analyzer and an automated pretreatment machine. Clin Chim Acta 2011;412:1767-72.
[20] Ito K, Kuno A, Ikehara Y, et al. LecT-Hepa, a glyco-marker derived from multiple lectins, as a predictor of liver fibrosis in chronic hepatitis C patients. Hepatology 2012, http://dx.doi.org/10.1002/hep.25815.
[21] Leevy CM. Diseases of the liver and biliary tract : standardization of nomenclature, diagnostic criteria, and prognosis. New York: Raven Press; 1994.
[22] Chang TT, Liaw YF, Wu SS, et al. Long-term entecavir therapy results in the reversal of fibrosis/cirrhosis and continued histological improvement in patients with chronic hepatitis B. Hepatology 2010;52:886-93.
[23] Wong VW, Vergniol J, Wong GL, et al. Diagnosis of fibrosis and cirrhosis using liver stiffness measurement in nonalcoholic fatty liver disease. Hepatology 2010;51: 454-62.
[24] Ganne-Carrie N, Ziol M, de Ledinghen V, et al. Accuracy of liver stiffness measurement for the diagnosis of cirrhosis in patients with chronic liver diseases. Hepatology 2006;44:1511-7.
[25] Bedossa P, Dargere D, Paradis V. Sampling variability of liver fibrosis in chronic hepatitis C. Hepatology 2003;38:1449-57.
[26] Colletta C, Smirne C, Fabris C, et al. Value of two noninvasive methods to detect progression of fibrosis among HCV carriers with normal aminotransferases. Hepatology 2005;42:838-45.
[27] Sagir A, Erhardt A, Schmitt M, Haussinger D. Transient elastography is unreliable for detection of cirrhosis in patients with acute liver damage. Hepatology 2008;47:592-5.
[28] Arena U, Vizzutti F, Corti G, et al. Acute viral hepatitis increases liver stiffness values measured by transient elastography. Hepatology 2008;47:380-4.

HEPATOLOGY
Official Journal of the American Association for the Study of Liver Diseases

LecT-Hepa, a Glyco-Marker Derived from Multiple Lectins, as a Predictor of Liver Fibrosis in Chronic Hepatitis C Patients

Kiyoaki Ito,[1] Atsushi Kuno,[2] Yuzuru Ikehara,[2] Masaya Sugiyama,[1] Hiroaki Saito,[1] Yoshihiko Aoki,[1] Teppei Matsui,[1] Masatoshi Imamura,[1] Masaaki Korenaga,[1] Kazumoto Murata,[1] Naohiko Masaki,[1] Yasuhito Tanaka,[3] Shuhei Hige,[4] Namiki Izumi,[5] Masayuki Kurosaki,[5] Shuhei Nishiguchi,[6] Michiie Sakamoto,[7] Masayoshi Kage,[8] Hisashi Narimatsu,[2] and Masashi Mizokami[1]

Assessment of liver fibrosis in patients with chronic hepatitis C (CHC) is critical for predicting disease progression and determining future antiviral therapy. LecT-Hepa, a new glyco-marker derived from fibrosis-related glyco-alteration of serum alpha 1-acid glycoprotein, was used to differentiate cirrhosis from chronic hepatitis in a single-center study. Herein, we aimed to validate this new glyco-marker for estimating liver fibrosis in a multicenter study. Overall, 183 CHC patients were recruited from 5 liver centers. The parameters *Aspergillus oryzae* lectin (AOL) / *Datura stramonium* lectin (DSA) and *Maackia amurensis* lectin (MAL)/DSA were measured using a bedside clinical chemistry analyzer in order to calculate LecT-Hepa levels. The data were compared with those of seven other noninvasive biochemical markers and tests (hyaluronic acid, tissue inhibitor of metalloproteases-1, platelet count, aspartate aminotransferase-to-platelet ratio index [APRI], Forns index, Fib-4 index, and Zeng's score) for assessing liver fibrosis using the receiver-operating characteristic curve. LecT-Hepa correlated well with the fibrosis stage as determined by liver biopsy. The area under the curve (AUC), sensitivity, and specificity of LecT-Hepa were 0.802, 59.6%, and 89.9%, respectively, for significant fibrosis; 0.882, 83.3%, and 80.0%, respectively, for severe fibrosis; and 0.929, 84.6%, and 88.5%, respectively, for cirrhosis. AUC scores of LecT-Hepa at each fibrosis stage were greater than those of the seven aforementioned noninvasive tests and markers. *Conclusion*: The efficacy of LecT-Hepa, a glyco-marker developed using glycoproteomics, for estimating liver fibrosis was demonstrated in a multicenter study. LecT-Hepa given by a combination of the two glyco-parameters is a reliable method for determining the fibrosis stage and is a potential substitute for liver biopsy. (HEPATOLOGY 2012;56:1448-1456)

Accurate staging of hepatic fibrosis in patients with chronic hepatitis C (CHC) is most important for predicting disease progression and determining the need for initiating antiviral therapy, such as interferon (IFN) therapy.[1,2] Liver biopsy has been considered the gold standard for fibrosis staging for many years.[3] However, liver biopsy is invasive and painful,[4,5] with rare but potentially life-threatening complications.[6] In addition, this method may suffer from sampling errors since only 1/50,000 of the organ is examined.[7] Furthermore, inter- and intraobserver discrepancies reaching levels of 10% to 20% have been

Abbreviations: α2-MG, α2-macroglobulin; AFP, alpha-fetoprotein; AGP, alpha-1 acid glycoprotein; ALT, alanine aminotransferase; AOL, Aspergillus oryzae *lectin; CHC, chronic hepatitis C; DSA,* Datura stramonium *lectin; GGT, gamma-glutamyltransferase; HA, hyaluronic acid; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; IFN, interferon; MAL,* Maackia amurensis *lectin; TIMP1, tissue inhibitors of metalloproteinases 1.*

From the [1]Research Center for Hepatitis and Immunology, National Center for Global Health and Medicine, Ichikawa, Japan; [2]Research Center for Medical Glycoscience, National Institute of Advanced Industrial Science and Technology, Tsukuba, Japan; [3]Department of Virology & Liver Unit, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan; [4]Department of Internal Medicine, Hokkaido University Graduate School of Medicine, Sapporo, Japan; [5]Gastroenterology and Hepatology, Musashino Red Cross Hospital, Tokyo, Japan; [6]Department of Internal Medicine, Hyogo College of Medicine, Nishinomiya, Japan; [7]Pathology, School of Medicine, Keio University, Japan; [8]Department of Pathology, Kurume University School of Medicine, Japan.

Received February 6, 2012; accepted April 22, 2012.

Supported by a grant (22-108) from the National Center for Global Health and Medicine in Japan and a grant from New Energy and Industrial Technology Development Organization of Japan.

reported using this method, leading to misdiagnosis of cirrhosis.[8] Therefore, finding a noninvasive method for diagnosing liver fibrosis is an emerging issue in the care of patients with CHC.

Several methods have been studied for the noninvasive diagnosis of hepatic fibrosis or cirrhosis, including clinical[9] or blood markers,[10,11] and signal analysis (ultrasonography, magnetic resonance imaging, and elastography).[12,13] Although each method can play a substantial role in the diagnosis of cirrhosis, it is evident that the best way of monitoring hepatitis progression employs an accurate serological method for the quantitative evaluation of fibrosis. We developed a new glyco-marker using multiple lectins that performed well in estimating liver fibrosis in a single-center study.[14,15]

Recent progress in glycoproteomics has had a great influence on work toward ideal, disease-specific biomarkers for a number of conditions. Glycoproteins that exhibit disease-associated glyco-alteration and are present in serum or other fluids have the potential to act as biomarkers for the diagnosis of a target disease,[16] because the features of glycosylation depend on the extent of cell differentiation and the stage of the cell. Detecting hepatic disease-associated glyco-markers for clinical applications has been a continuous challenge since the early 1990s, because increased fucosylation on complex-type *N*-glycans has been frequently detected in glycoproteins from patients with hepatocellular carcinoma (HCC) and cirrhosis.[17,18] Of all the alpha-fetoprotein (AFP) glycoforms, more than 30% have been found to react to a fucose-binding lectin, *Lens culinaris* agglutinin. This fraction, designated AFP-L3, was approved by the U.S. Food and Drug Administration (FDA) in 2005 for the diagnosis and prognosis of HCC.[19] We have found that two fibrosis-indicator lectins (*Aspergillus oryzae* lectin [AOL] and *Maackia amurensis* lectin [MAL]) together with an internal, standard lectin (*Datura stramonium* lectin [DSA]) on an alpha 1-acid glycoprotein (AGP) could, using lectin microarray, clearly distinguish between cirrhosis and chronic hepatitis patients.[14] We have further simplified this quantitative method so that it could be performed using bedside, clinical chemistry analyzers.[15]

The aim of the current study was to evaluate this new glyco-marker (LecT-Hepa) using multiple lectins and bedside clinical chemistry analyzers for use in the assessment of liver fibrosis. In this multicenter study we compared the method's efficiency in estimating liver fibrosis with other noninvasive fibrosis markers and tests.

Materials and Methods

Study Population. This study included 183 consecutive adult patients with CHC who had undergone percutaneous liver biopsy at one of the following institutions: Hokkaido University Hospital, Musashino Red Cross Hospital, National Center for Global Health and Medicine, Hyogo College of Medicine Hospital, or Nagoya City University Hospital in Japan. A diagnosis of CHC was defined as detectable serum anti-hepatitis C virus (HCV) antibody and HCV-RNA, found using polymerase chain reaction assays, of at least 2 points. Exclusion criteria were coinfection with hepatitis B virus or human immunodeficiency virus (HIV), and other disorders that commonly cause liver diseases. Informed consent was obtained from each patient who participated in the study. This study was conducted in accordance with the provisions of the Declaration of Helsinki and was approved by our Institutional Review Board.

Histological Staging. Ultrasonography-guided liver biopsy was performed according to a standardized protocol. Specimens were fixed, paraffin-embedded, and stained with hematoxylin-eosin and Masson's trichrome. A minimum of six portal tracts in the specimen were required for diagnosis. All liver biopsy samples were independently evaluated by two senior pathologists who were blinded to the clinical data. Liver fibrosis stages were assessed using METAVIR fibrosis (F) staging.[20] Significant fibrosis was defined as METAVIR F $\geq$2, severe fibrosis as METAVIR F $\geq$3, and cirrhosis as METAVIR F4. Two patients were excluded from the study because of inadequate histological samples.

Clinical and Biological Data. The age and sex of the patients were recorded. Serum samples were collected immediately before or no more than 2 months

Address reprint requests to: Masashi Mizokami, M.D., Ph.D., Research Center for Hepatitis and Immunology, National Center for Global Health and Medicine, 1-7-1, Konodai, Ichikawa 272-8516, Japan. E-mail: mmizokami@hospk.ncgm.go.jp; fax: +81-(0)47-375-4766.

View this article online at wileyonlinelibrary.com.

DOI 10.1002/hep.25815

Potential conflict of interest: Nothing to report.

after liver biopsy and were stored at −80°C until analysis. The concentrations of the following variables were obtained by analyzing the serum samples: aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), total bilirubin, albumin, cholinesterase, total cholesterol, platelet count (platelets), prothrombin time, haptoglobin, hyaluronic acid (HA), α2-macroglobulin (α2-MG), tissue inhibitors of metalloproteinases 1 (TIMP1). The aspartate aminotransferase-to-platelet ratio index (APRI), Fib-4 index, Forns index, and Zeng's score were calculated according to published formulae appropriate to each measure.[2,7,21,22]

Rapid Lectin-Antibody Sandwich Immunoassay Using HISCL. Fibrosis-specific glyco-alteration of AGP was qualified from simultaneous measurements of the lectin-antibody sandwich immunoassays using three lectins (DSA, MAL, and AOL). In principle, the glycan part of the AGP was captured by the lectin immobilized on the magnetic beads, and the captured AGP was then quantified by an antihuman AGP mouse monoclonal antibody probe that was cross-linked to an alkaline phosphatase (ALP-αAGP). The assay manipulation was fully automated using a chemiluminescence enzyme immunoassay machine (HISCL-2000i; Sysmex, Kobe, Japan). We used the following criterion formula, named the "LecT-Hepa Test," to enhance the diagnostic accuracy by combining two glyco-parameters (AOL/DSA and MAL/DSA) as described before: $F = \mathrm{Log}_{10}[\mathrm{AOL/DSA}]*8.6-[\mathrm{MAL/DSA}]$.[15]

Statistical Analyses. Quantitative variables were expressed as the mean ± standard deviation (SD) unless otherwise specified. Categorical variables were compared using a chi-squared test or Fisher's exact test, as appropriate, and continuous variables were compared using the Mann-Whitney U test. $P < 0.05$ was considered statistically significant. A multivariate forward stepwise logistic regression analysis was performed to determine the independent predictors of the absence or presence of significant fibrosis, severe fibrosis, and cirrhosis, respectively. Pearson's correlation coefficient was used as necessary. To assess the classification efficiencies of various markers for detecting significant fibrosis, severe fibrosis, and cirrhosis,[23] and to determine area under the curve (AUC) values, receiver-operating characteristic (ROC) curve analysis was also carried out. Diagnostic accuracy was expressed as the diagnostic specificity (specificity), diagnostic sensitivity (sensitivity), positive predictive values (PPV), negative predictive values (NPV), positive likelihood ratio (LR [+]), negative likelihood ratio (LR [−]), and AUC (95% confidence interval [95% CI]). We performed statistical analyses using STATA v. 11.0 (StataCorp, College Station, TX).

Table 1. Baseline Characteristics of the 183 Patients with Chronic Hepatitis C at the Time of Liver Biopsy

Features	Total (n = 183)
Age (years)	57.6 ± 11.4
Male sex	75 (41.0)
AST (IU/L)	57.4 ± 43.9
ALT (IU/L)	62.8 ± 56.8
GGT (IU/L)	51.1 ± 62.6
Bilirubin (mg/dL)	0.7 ± 0.4
Albumin (g/L)	4.1 ± 0.4
Cholinesterase (IU/L)	283.5 ± 97.0
Cholesterol (mg/dL)	174.1 ± 35.5
Platelets (10^9/L)	163 ± 57
Prothrombin time (%)	87.2 ± 33.4
α2-MG (g/L)	356.8 ± 133.1
HA (μg/L)	205.3 ± 428.0
TIMP1 (pg/ml)	210.6 ± 87.7
AOL/DSA	6.3 ± 12.3
MAL/DSA	9.0 ± 3.1
Fibrosis stage (%):	
F0-1	89 (48.6)
F2	46 (25.1)
F3	22 (12.0)
F4	26 (14.2)

Results

Baseline Characteristics of the 183 Patients with Chronic Hepatitis C at the Time of Liver Biopsy. Patient characteristics at the time of liver biopsy are shown in Table 1. The mean age of the 183 patients was 57.6 ± 11.4 years, and 75 (41%) of them were men. F0-F1 was diagnosed in 89 cases (48.6%), F2 in 46 (25.1%), F3 in 22 (12.0%), and F4 (cirrhosis) in 26 (14.2%).

Comparison of Variables Associated with the Presence of Significant Fibrosis by Univariate and Multivariate Analysis. Variables associated with the presence of significant fibrosis were assessed by univariate and multivariate analysis (Table 2). The variables of age ($P = 0.001$), AST ($P < 0.0001$), ALT ($P < 0.0001$), GGT ($P < 0.0001$), bilirubin ($P = 0.014$), α2-MG ($P = 0.002$), HA ($P < 0.0001$), TIMP1 ($P < 0.0001$), and AOL/DSA ($P < 0.0001$) were significantly higher in the significant fibrosis group than in the not significant fibrosis group. The variables albumin ($P < 0.001$), cholinesterase ($P < 0.0001$), cholesterol ($P = 0.005$), platelets ($P < 0.0001$), prothrombin time ($P = 0.0001$), and MAL/DSA ($P < 0.0001$) were significantly lower in the significant fibrosis group than in the not significant fibrosis group. Multivariate analysis showed that platelets (odds ratio [OR]: 0.87,