assessed according to Brunt's criteria [32]. Significant and severe fibrosis was defined as ≥stage 2 and ≥stage 3, respectively. Thirty-six patients were excluded because of clinical and/or histological reasons; thus, 289 patients were included in the final analysis.

Clinical and biochemical data

Relevant clinical data were recorded, including the patient's age, sex, weight, and height. Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. Venous blood samples were obtained in the morning after overnight fasting, either immediately before or no more than 2 months after liver biopsy. The blood samples were stored at −80 °C until analysis.

The biochemical variables were measured using a conventional automated analyzer at the respective hospitals. We analyzed the serum levels for the following: platelet count, prothrombin time, bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltranspeptidase, albumin, cholesterol, triglyceride, fasting plasma glucose (FPG), ferritin, and hyaluronic acid. The AST-to-platelet ratio index (APRI) was calculated as follows: [AST (U/L)/UNL × 100]/platelet count. In this equation, UNL is the upper limit of the normal AST [33]. The FIB-4 index was calculated as follows: age (years) × AST (U/L)/platelet count ($\times 10^9$/L) × $\sqrt{\text{ALT}}$ (U/L) [20]. The NAFLD fibrosis score was calculated as follows: −1.675 + 0.037 × age (years) + 0.094 × BMI (kg/m^2) + 1.13 × impaired fasting glycemia or diabetes (yes = 1; no = 0) + 0.99 × AST/ALT ratio − 0.013 × platelet ($\times 10^9$/L) − 0.66 × albumin (g/dL) [21].

Serum *Wisteria floribunda* agglutinin-positive Mac-2 binding protein value

The WFA$^+$-M2BP value in sera was measured by a WFA-antibody immunoassay using a chemiluminescence enzyme immunoassay machine (HISCL-2000i; Sysmex, Kobe, Japan), as previously reported [27, 28, 30, 31]. The measured values of WFA$^+$-M2BP using the conjugated WFA were indexed with the obtained values using the following equation: cutoff index (COI) = ([WFA$^+$-M2BP]$_{sample}$ − [WFA$^+$-M2BP]$_{NC}$) 7 ([WFA$^+$-M2BP]$_{PC}$) − [WFA$^+$-M2BP]$_{NC}$). In this equation, [WFA$^+$-M2BP]$_X$ denotes the [WFA + -M2BP] count of the serum sample ($_X$ = sample), positive control ($_X$ = PC), or negative control ($_X$ = NC).

Statistical analysis

Quantitative values are presented as mean ± standard deviation, unless otherwise noted. The Steel–Dwass test was used for multiple comparisons of continuous variables among the different groups. Univariate and multivariate analyses were performed using a logistic regression model. Each cutoff value was determined from the receiver operating characteristic (ROC) curve analyses. The diagnostic performances of the markers were expressed as the diagnostic specificity, sensitivity, positive predictive value, negative predictive value, and area under the ROC (AUROC) curve. p values <0.05 were considered statistically significant. All statistical analyses were performed using JMP, version 11 software (SAS Institute, Tokyo, Japan).

Results

Cross-sectional association between *Wisteria floribunda* agglutinin-positive Mac-2 binding protein values and the fibrosis stage

The patients' characteristics are summarized in Table 1. The mean age of the 289 patients (159 men and 130 women) was 54.8 ± 14.6 years old. Figure 1 shows the serum WFA$^+$-M2BP values for each fibrosis stage. The serum WFA$^+$-M2BP values measured by glycan-based immunoassay ranged from 0.12 to 11.06 (COI). The

Table 1 Patients' clinical characteristics and laboratory data

Features	Total (n = 289)
Male/female	159/130
Age (years)	54.8 ± 14.6
Body mass index (kg/m^2)	27.6 ± 4.7
Platelet count (10^9/l)	18.9 ± 6.8
Prothrombin time (%)	99.3 ± 16.7
Bilirubin (mg/dl)	0.97 ± 0.6
AST (U/l)	61.4 ± 48.9
ALT (U/l)	85.5 ± 68.9
GGT (U/l)	92.3 ± 89.9
Albumin (g/dl)	4.2 ± 0.4
Cholesterol (mg/dl)	195.4 ± 41.1
Triglyceride (mg/dl)	144.4 ± 77.2
FPG (mg/dl)	115.2 ± 38.4
Ferritin (ng/ml)	261.2 ± 258.5
WFA$^+$-M2BP (COI)	1.26 ± 1.44
Fibrosis stage (0/1/2/3/4)	35/113/49/41/51

Values are expressed as mean ± standard deviation

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *COI* cutoff index, *GGT* gamma-glutamyl transpeptidase, *FPG* fasting plasma glucose, WFA$^+$-*M2BP Wisteria floribunda* agglutinin-positive Mac-2 binding protein

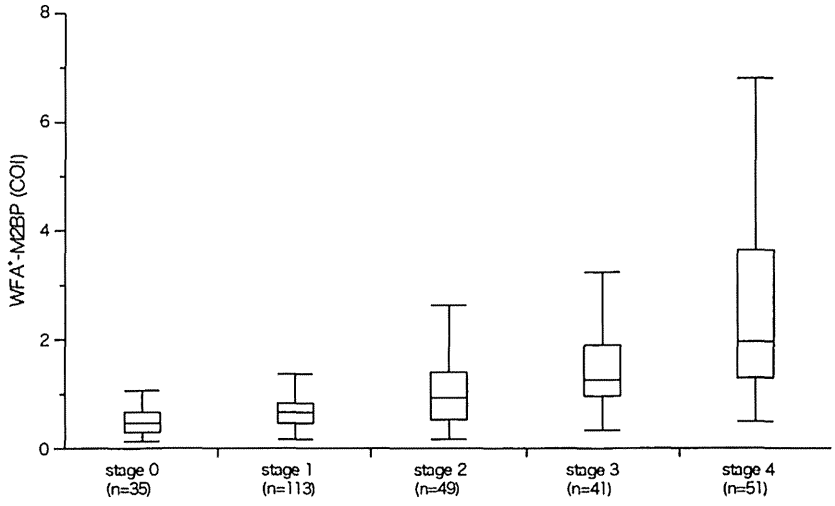

Fig. 1 The serum *Wisteria floribunda* agglutinin-positive Mac-2 binding protein (WFA^+-M2BP) values for each fibrosis stage. The *top* and *bottom* of each *box* represent the first and third quartiles, respectively, with the height of the box representing the interquartile range, covering 50 % of the values. The *line* across each *box* represents the median. The *whiskers* show the highest and lowest values. All pairs of groups are significantly different, as assessed using the Steel–Dwass test ($p < 0.01$). *COI* cutoff index

Table 2 Variables associated with the fibrosis stage according to multivariate regression analyses

	Stage 0 vs. stages 1–4		Stages 0–1 vs. stages 2–4		Stages 0–2 vs. stages 3–4		Stages 0–3 vs. stage 4	
	Odds ratio (95 % CI)	*p* value	Odds ratio (95 % CI)	*p* value	Odds ratio (95 % CI)	*p* value	Odds ratio (95 % CI)	*p* value
Age (years)			1.049 (1.014–1.087)	0.006				
BMI (kg/m^2)	1.228 (1.089–1.412)	0.002						
Platelet count (10^9/L)					0.864 (0.787–0.941)	0.001	0.895 (0.814–0.978)	0.017
Prothrombin time (%)	0.948 (0.914–0.982)	0.004	0.957 (0.925–0.986)	0.007			0.963 (0.927–0.993)	0.028
AST (U/l)	1.078 (1.023–1.144)	0.008	1.036 (1.022–1.052)	<0.001				
FPG (mg/dl)			1.013 (1.004–1.024)	0.007	1.014 (1.004–1.024)	0.004	1.012 (1.002–1.022)	0.013
WFA^+-M2BP (COI)			5.875 (2.339–16.369)	<0.001	8.471 (3.562–22.725)	<0.001	2.390 (1.463–4.423)	0.002

CI confidence interval, *BMI* body mass index, *AST* aspartate aminotransferase, *FPG* fasting plasma glucose, WFA^+-*M2BP Wisteria floribunda* agglutinin-positive Mac-2 binding protein, *COI* cutoff index

WFA^+-M2BP value in patients with stages 0 ($n = 35$), 1 ($n = 113$), 2 ($n = 49$), 3 ($n = 41$), and 4 ($n = 51$) of fibrosis had COIs of 0.57, 0.70, 1.02, 1.57, and 2.96, respectively, demonstrating a stepwise increase with an increasing severity of liver fibrosis (Fig. 1). All pairs of groups differed significantly according to the Steel–Dwass test (stage 0 vs. stage 1, $p = 0.012$; stage 0 vs. stage 2, $p < 0.001$; stage 0 vs. stage 3, $p < 0.001$; stage 0 vs. stage 4, $p < 0.001$; stage 1 vs. stage 2, $p = 0.002$; stage 1 vs. stage 3, $p < 0.001$; stage 1 vs. stage 4, $p < 0.001$; stage 2 vs. stage 3, $p = 0.014$; stage 2 vs. stage 4, $p < 0.001$; and stage 3 vs. stage 4, $p = 0.008$).

Comparisons of variables associated with the diagnosis of the fibrosis stage

The variables associated with each stage of liver fibrosis were assessed by univariate and multivariate analyses (Tables S1, 2).

Variables associated with the presence of fibrosis (≥stage 1)

According to univariate analysis, eight variables (age, BMI, platelet count, prothrombin time, AST, ALT,

albumin, and the WFA$^+$-M2BP value) were associated with the presence of fibrosis (Table S1). Multivariate analysis showed that the BMI [odds ratio (OR) 1.228, 95 % confidence interval (CI) 1.089–1.412], prothrombin time (OR 0.948; 95 % CI 0.914–0.982), and AST (OR 1.078; 95 % CI 1.023–1.144) were independently associated with the presence of fibrosis (Table 2).

Variables associated with the presence of significant fibrosis (≥stage 2)

Univariate analysis identified ten variables (sex, age, platelet count, prothrombin time, bilirubin, AST, albumin, cholesterol, FPG, and the WFA$^+$-M2BP value) that were associated with the presence of significant fibrosis (Table S1). However, multivariate analysis showed that age (OR 1.049; 95 % CI 1.014–1.087), prothrombin time (OR 0.957; 95 % CI 0.925–0.986), AST (OR 1.036; 95 % CI 1.022–1.052), ALT (OR 1.036; 95 % CI 1.022–1.052), FPG (OR 1.013; 95 % CI 1.004–1.024), and the WFA$^+$-M2BP value (OR 5.875; 95 % CI 2.339–16.369) were independently associated with the presence of significant fibrosis (Table 2).

Variables associated with the presence of severe fibrosis (≥stage 3)

According to univariate analysis, ten variables (sex, age, platelet count, prothrombin time, bilirubin, albumin, cholesterol, triglyceride, FPG, and the WFA$^+$-M2BP value) were associated with the presence of severe fibrosis (Table S1). However, multivariate analysis showed that the platelet count (OR 0.864; 95 % CI 0.787–0.941), FPG (OR 1.014; 95 % CI 1.004–1.024), and the WFA$^+$-M2BP value (OR 8.471; 95 % CI 3.562–22.725) were independently associated with the presence of severe fibrosis (Table 2).

Variables associated with the presence of cirrhosis (stage 4)

Univariate analysis identified 11 variables (sex, age, platelet count, prothrombin time, bilirubin, ALT, albumin, cholesterol, triglyceride, FPG, and the WFA$^+$-M2BP value) that were associated with the presence of cirrhosis (Table S1). Multivariate analysis identified that the platelet count (OR 0.895; 95 % CI 0.814–0.978), prothrombin time (OR 0.963; 95 % CI 0.927–0.993), FPG (OR 1.012; 95 % CI 1.002–1.022), and the WFA$^+$-M2BP value (OR 2.390; 95 % CI 1.462–4.423) were independently associated with the presence of cirrhosis (Table 2).

Diagnostic power of the *Wisteria floribunda* agglutinin-positive Mac-2 binding protein values for each fibrosis stage

The WFA$^+$-M2BP ROC curves for diagnosing each fibrosis stage are presented in Fig. 2. The AUROC curve values (95 % CI) for the prediction of ≥stage 1, ≥stage 2, ≥stage 3, and stage 4 using the serum WFA$^+$-M2BP values were 0.788 (0.736–0.833), 0.838 (0.790–0.879), 0.876 (0.832–0.911), and 0.879 (0.835–0.914), respectively (Table 3). The optimal cutoff values were 0.59 for ≥stage 1, 0.90 for ≥stage 2, 0.94 for ≥stage 3, and 1.46 for stage 4 (Table 3). The sensitivities for the prediction of ≥stage 1, ≥stage 2, ≥stage 3, and stage 4 were 74.8, 77.3, 85.9, and 72.6 %, respectively; whereas, the specificities were 74.3, 81.1, 74.6, and 87.0 %, respectively (Table 3).

Comparisons of AUROC curve values for diagnosing the fibrosis stage

The AUROC curve values for diagnosing each fibrosis stage are shown in Table 4. Compared with the other surrogate markers and scoring systems, the serum WFA$^+$-M2BP was the most useful marker for differentiating stages 0–2 from stages 3–4 and stages 0–3 from stage 4. The AUROC curve values for differentiating stages 0–1 from stages 2–4 were compatible with the serum WFA$^+$-M2BP (0.838), hyaluronic acid (0.833), and the FIB-4 index (0.844).

Discussion

Clinically, it is very important to identify patients who have NASH with advanced fibrosis, because these patients have more liver-related complications and a greater mortality rate than patients who have NASH without liver fibrosis [4–7]. Although a liver biopsy is the gold standard for diagnosing and assessing the stages of fibrosis, research on noninvasive methods for assessing the fibrosis stages have rapidly evolved over the last decade [17–26]. In this study, we found that the serum WFA$^+$-M2BP values measured using a glycan-based immunoassay provided a useful diagnostic factor for assessing the liver fibrosis stage in NAFLD patients (Fig. 1). The glycan-based immunoassay was previously developed as a simple system for automatically detecting unique fibrosis-related glycoalterations [27–31]. Moreover, the accuracy of the serum WFA$^+$-M2BP values for diagnosing severe fibrosis and cirrhosis was superior to that offered by other surrogate markers and tests (Table 4).

M2BP is a secreted glycoprotein that is found in the serum of healthy individuals, but its concentration

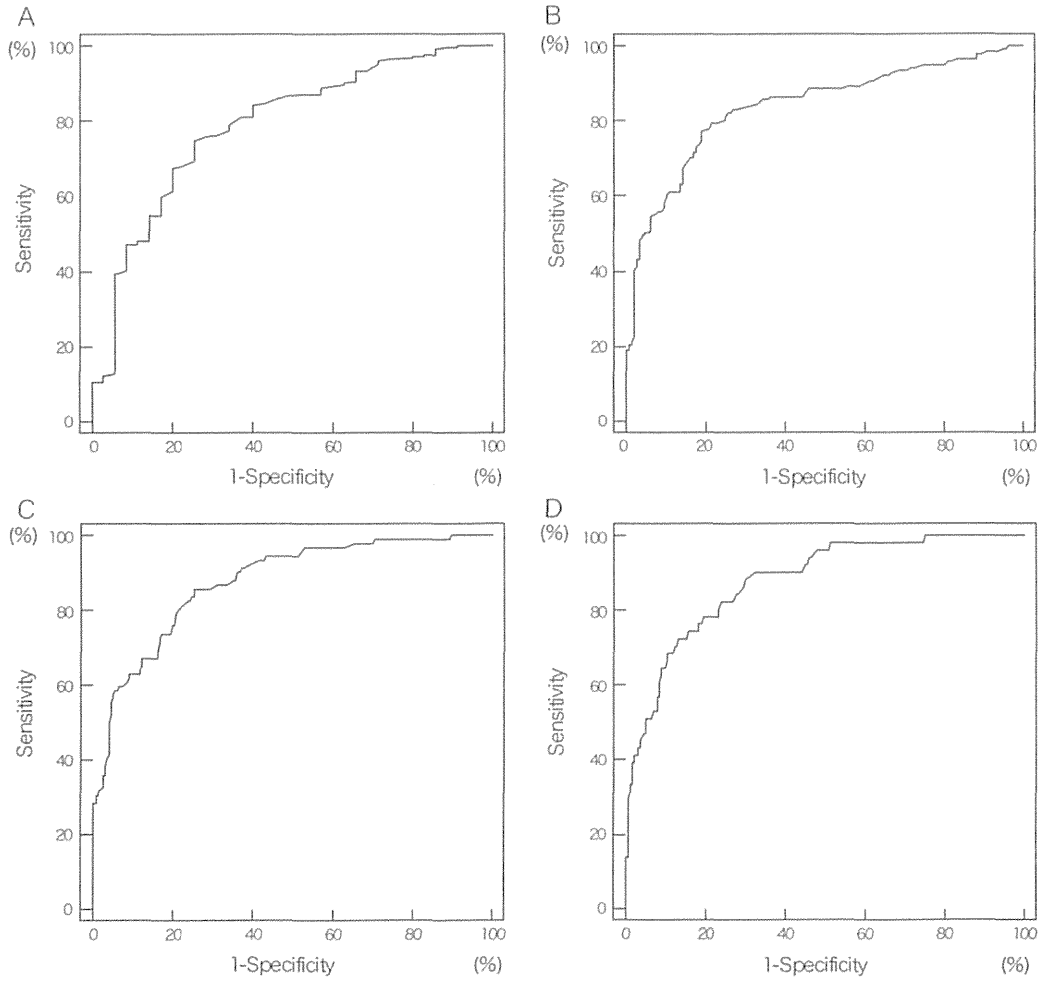

Fig. 2 The diagnostic capabilities of the serum *Wisteria floribunda* agglutinin-positive Mac-2 binding protein (WFA^+-M2BP) values for assessing the stage of liver fibrosis. The areas under the receiver operating characteristic curve of serum WFA^+-M2BP for diagnosing liver fibrosis were as follows: **a** 0.788 for stage ≥1; **b** 0.838 for stage ≥2; **c** 0.876 for stage ≥3; and **d** 0.879 for stage 4

increases in patients with various cancers and viral infections, including HCV [34, 35]. This protein binds galectin-3, β-1 integrins, collagens, and fibronectin and has some relevance to cell–cell and cell–extracellular matrix adhesion [36, 37]. Therefore, it is reasonable to assume that M2BP reflects the progression of fibrosis in cases of CLD. Indeed, using proteome analysis, Cheung et al. [38] found that serum M2BP is a potential marker of fibrosis progression in HCV patients.

In this study, we found that the serum WFA^+-M2BP value can be used to distinguish the fibrosis stages in NAFLD patients (Fig. 1; Tables S1, 2, 3). Recently, Kamada et al. [39] reported that the serum M2BP value (the whole M2BP protein measured by enzyme-linked immunosorbent assay) can be used for predicting the fibrosis stage in NAFLD patients. However, there are several differences between the present study and Kamada et al.'s study. In our study, the serum WFA^+-M2BP value (the altered M2BP with fibrosis-related *N*-glycans measured by glycan-based immunoassay) increased stepwise with the increasing severity of liver fibrosis, whereas a stepwise increase was not found in Kamada et al.'s study. Further, our method can distinguish between the fibrosis stages more clearly, not only in patients with advanced fibrosis stage but also in those with earlier fibrosis stages of NAFLD (Fig. 1). In our previous study [27], we found that both the quantity and quality of M2BP were altered during the progression of fibrosis of CLD due to HCV. Since the *N*-glycosylation of M2BP was dramatically altered during the progression of liver fibrosis, we considered that the

Table 3 Serum *Wisteria floribunda* agglutinin-positive Mac-2 binding protein values for assessing liver fibrosis

Stage	AUC (95 % CI)	Cutoff level	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Predictive accuracy (%)
≥Stage 1	0.788 (0.736–0.833)	0.59	74.8	74.3	95.5	28.9	74.7
≥Stage 2	0.838 (0.790–0.879)	0.90	77.3	81.1	79.6	78.9	79.2
≥Stage 3	0.876 (0.832–0.911)	0.94	85.9	74.6	61.2	91.9	78.2
Stage 4	0.879 (0.835–0.914)	1.46	72.6	87.0	54.4	93.7	84.4

AUC area under receiver operating characteristic curve, *CI* confidence interval, *PPV* positive predictive value, *NPV* negative predictive value

Table 4 Comparisons of the areas under the receiver operating characteristic curves for each fibrosis marker and scoring system

Marker and score	≥Stage 1	≥Stage 2	≥Stage 3	Stage 4
WFA^+-M2BP	0.788	0.838	0.876	0.879
Platelet count	0.649	0.719	0.810	0.815
Hyaluronic acid	0.757	0.833	0.856	0.858
AST/ALT ratio	0.607	0.733	0.770	0.752
APRI	0.867	0.804	0.758	0.745
FIB-4 index	0.793	0.844	0.857	0.849
NAFLD fibrosis score	0.766	0.811	0.808	0.824

WFA^+-*M2BP Wisteria floribunda* agglutinin-positive Mac-2 binding protein, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *APRI* AST-to-platelet ratio index, *NAFLD* non-alcoholic fatty liver disease

WFA^+-M2BP reflects the fibrosis status more precisely than the whole M2BP protein. Further, the quantification of the WFA^+-M2BP may offer a better marker for assessing the liver fibrosis stage than does the quantification of the M2BP protein. Currently, the *N*-glycan structures of WFA^--M2BP and WFA^+-M2BP are being analyzed using mass spectrometry in our laboratory. Moreover, our system has been converted to a fully automated immunoassay analyzer for clinical use, featuring a measurement time of only 17 min, which has clear practical implications [27, 28, 30, 31].

Numerous non-invasive panels of the tests have been developed to assess the liver fibrosis stages [17–26]. In this study, the serum WFA^+-M2BP values offered a superior AUROC curve for the diagnosis of severe fibrosis and cirrhosis compared with the FIB-4 index and five other markers and scoring systems (Table 4). In a study of a large Japanese cohort, the FIB-4 index was the most useful index for diagnosing patients with advanced fibrosis [40]. Although the American Association for the Study Liver Diseases' guidelines [3] recommend the NAFLD fibrosis score [21] when deciding whether to perform a liver biopsy, the usefulness of this score remains questionable in Asian patients [40, 41]. Consistent with these studies, the NAFLD fibrosis score yielded lower AUROCs than the WFA^+-M2BP values and the FIB-4 index for diagnosing fibrosis in our cohort (Table 4).

There are two main strengths of the present study's cohort. First, the sample size ($n = 289$) was relatively large, and the patients' clinical backgrounds were well characterized. Second, the pathological diagnoses were performed and validated by three experienced liver-specific pathologists. Currently, the definitive diagnosis of NAFLD and the distinction of its phenotypes rely on the pathologist's interpretation of the liver biopsy [8]; therefore, an accurate and reproducible consensus regarding the pathological findings is necessary for diagnosing NAFLD. However, in practice, the interpretation of NAFLD's histology varies substantially. In this study, we excluded patients whose liver samples were inadequate for histological evaluation (e.g., because of insufficient sample size). Moreover, the considerable rate of inter-observer variation is one of the major problems in the histological diagnosis of NAFLD [13–17]. Our strategy mainly focused on reducing this variation, and our study may provide a reliable cohort for identifying surrogate markers and for investigating the management of NAFLD patients.

This study also has several limitations. First, we investigated the usefulness of the serum WFA^+-M2BP values in a cross-sectional study. Therefore, the use of the serum WFA^+-M2BP values for monitoring natural history, predicting outcomes, and predicting responses to therapeutic interventions remain unknown. In fact, the prevalence of NAFLD is high among individuals with diabetes or dyslipidemia [1–3], and some patients have already managed their condition through lifestyle interventions and/or medication at the time of liver biopsy. Further prospective studies are necessary to address these issues. In addition, since the biochemical analyses were performed separately at the respective hospitals, any variations among each institution cannot be ruled out. Moreover, several selection

biases may be present, because all the patients had been diagnosed and had received liver biopsies at hepatology centers, which may have caused referral bias. Therefore, validation studies are necessary in the general population.

In conclusion, the measurement of the serum WFA^{+}-M2BP values using a glycan-based immunoassay provides an accurate and reliable method for assessing the liver fibrosis stage in NAFLD patients. This method appears quite promising as a means for evaluating the natural course of the disease, therapeutic effects, and the suitability of liver biopsies.

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Conflict of interest The authors declare that they have no conflict of interest.

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＜原　著＞

大学病院の非肝臓内科におけるHBs抗原およびHCV抗体陽性者に対する肝疾患診療の実態

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要旨：ウイルス性肝炎対策において，医療機関における肝臓内科と非肝臓内科間の院内連携の実態は不明である．そこで，当院における非肝臓内科でのHBs抗原およびHCV抗体の測定状況，ならびに陽性者における肝炎診療の実態を明らかにすることを目的に，本研究を実施した．2010年1月～12月，肝臓内科以外の27診療科でHBs抗原またはHCV抗体を測定した症例（重複例を除外，HBs抗原6,648例，HCV抗体6,612例）を対象とした．HBs抗原陽性126例（1.9%），推定B型慢性肝炎66例（1.0%），HCV抗体陽性487例（7.4%），推定HCVキャリア369例（5.6%），推定C型慢性肝炎244例（3.7%）であった．高い感染率にもかかわらず，HBVキャリアの79%，HCVキャリアの82%は肝臓内科との連携がなく，そのうち，前者の89%，後者の97%においてウイルス性肝炎に関する診療方針の記載もみられなかった．肝炎ウイルスキャリア率が高く，かつ肝臓専門医を擁する医療機関において，適切な肝炎診療を行うための院内連携システムの構築が早急に必要である．

索引用語：肝炎ウイルス　HBs抗原　HCV抗体　スクリーニング　院内連携システム

緒　言

我が国におけるHCV感染者は，諸外国に比して高齢化が進んでおり，肝癌発症のリスクが高いことが指摘されている[1)]．国内における肝癌死亡率はHCV感染率に一致して西高東低の分布を示すが，なかでも佐賀県は全国有数のC型肝炎高罹患地区であり，非常に高い肝癌死亡率を有している[2)~4)]．平成23年度の肝および肝内胆管の悪性新生物の粗死亡率（10万人対）は全国の25.3に対し佐賀県は44.0であり[5)]，依然，全国ワースト1位の状況が続いている．B型肝炎については，C型肝炎ほど全国的な罹患率の差はないが，佐賀県は比較的高率である[6)]．佐賀県のこれまでの検診データより，既に肝癌死亡は減少に転じてきているものの，今後10年間は依然として現在の全国平均よりも高い肝癌死亡率が予想されており，更なるウイルス性肝炎対策が急務である[7)]．

肝炎対策の問題点として，検診での肝炎ウイルス検査受検率が低いこと，そのうち要精密と判断された受検者の医療機関受診率が低いこと，また，たとえ医療機関を受診しても必ずしも適切な医療が提供されていないこと等が既に指摘されており，各方面で対策が講じられつつある[8)9)]．一方で，大学病院などの医療機関においては，術前スクリーニング等で肝炎ウイルス検査を行う機会が多いが，その陽性者が適切な肝炎診療を受けているかは不明である．平成23年5月16日に厚生労働省から出された「肝炎対策の推進に関する基

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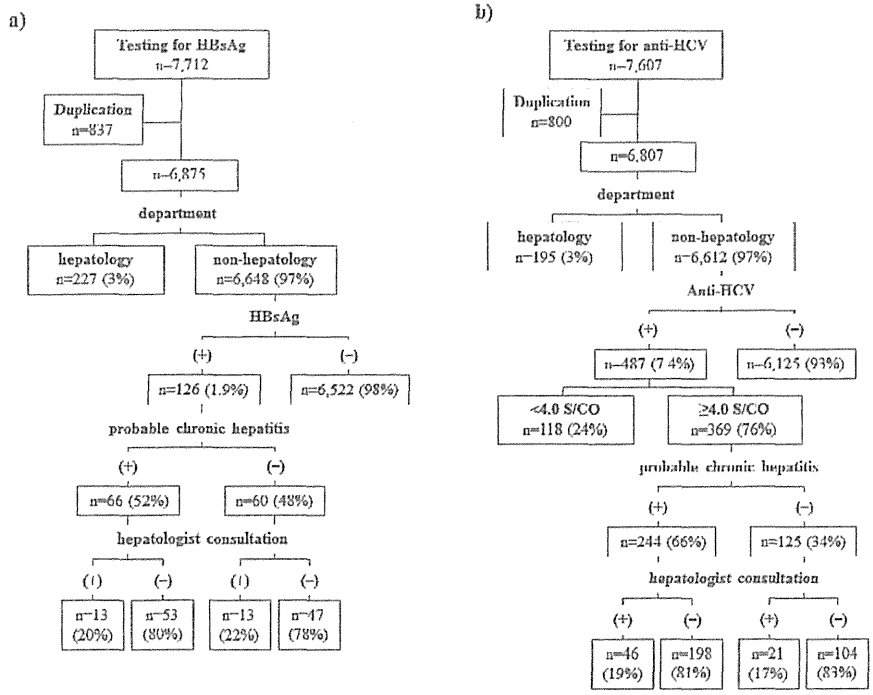

Fig. 1　Flow chart of the study subjects who underwent screening tests for a) HBsAg and b) anti-HCV.

本的な指針」[10]では，国および地方公共団体が医療機関に対し，手術前等に行われる肝炎ウイルス検査の結果について受検者に適切に説明を行うよう要請されており，その実態調査を行うことが目標として掲げられている．

そこで，大学病院における肝臓内科以外でのHBs抗原およびHCV抗体の測定状況，ならびに陽性者への肝炎診療の実態を明らかにすることを目的に，本研究を実施した．

対象および方法

2010年1月から12月まで，佐賀大学医学部附属病院にてHBs抗原（HBsAg：hepatitis B surface antigen）またはHCV抗体(anti-HCV：anti-hepatitis C virus antibody)検査を実施された症例（HBsAg 7,712例，anti-HCV 7,607例）のうち，重複例を除き肝臓内科以外の27診療科（非肝臓内科）で測定された症例（HBsAg 6,648例，anti-HCV 6,612例）を対象とした（Fig. 1）．HBsAgはARCHITECT-HBsAg QT（Abbott Japan, Tokyo, Japan）で測定し，0.05 IU/ml以上を陽性と判定した．Anti-HCVはARCHITECT-HCV（Abbott Japan, Tokyo, Japan）で測定し，1.0 S/CO以上を陽性と判定し，1.0～4.0 S/COを低力価，4.0～12.0 S/COを中力価，12.0 S/CO以上を高力価と判別した[11]．HBsAg陽性例においては，血小板数$<15\times10^4/\mu L$，AST≦ALT，ALT≧31 IU/Lのいずれかを満たす症例は慢性肝炎の可能性があるものと仮定し，推定B型慢性肝炎と定義した．Anti-HCV陽性例においては，中・高力価をHCVキャリアと仮定し，そのうち血小板数$<15\times10^4/\mu L$，AST≦ALT，ALT≧31 IU/Lのいずれかを満たす症例を推定C型慢性肝炎と定義した．

ウイルスキャリアにおいて肝臓内科へのコンサルトがあった群（介入群）となかった群（非介入群）の背景因子を比較し，有意差検定はMann-Whitney U検定，χ^2検定またはFisherの直接確率検定を用い，$p<0.05$を有意とした．ウイルスキャリアにおいて肝臓内科へのコンサルトに寄与する因子について，単変量および多変量のロジスティック回帰分析を施行した．多変量

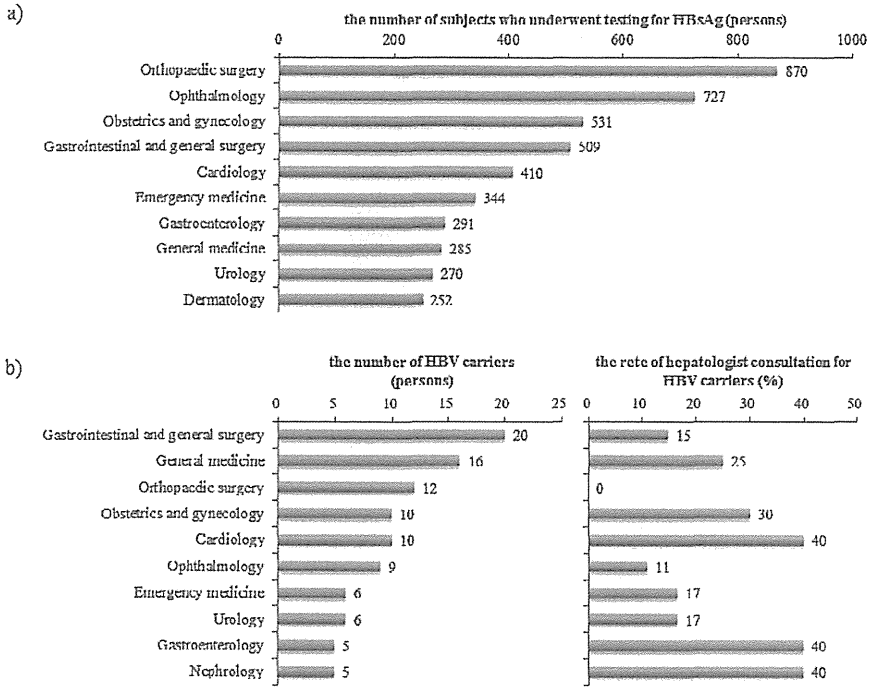

Fig. 2　a) The number of subjects who underwent HBsAg screening test in the top 10 non-hepatology departments. b) The number of HBV carriers in the top 10 non-hepatology departments, and the rate of hepatologist consultation.

解析の因子は，単変量解析で $p<0.15$ の因子を選択した．診療科は内科系（総合診療科，消化器内科，循環器内科，腎臓内科，呼吸器内科，リウマチ・膠原病内科，血液・腫瘍内科，神経内科，感染症内科）と非内科系（一般・消化器外科，心臓血管外科，呼吸器外科，脳神経外科，整形外科，形成外科，眼科，耳鼻咽喉科，産婦人科，泌尿器科，皮膚科，歯科口腔外科，精神科，小児科，麻酔科，救急科，放射線科，検査科）に分類した．また，同様の解析を，肝炎の有無別に層別化して施行した．

本研究は佐賀大学医学部附属病院の倫理委員会の許可を得て実施し，対象者の個人情報保護に十分配慮した上でデータの抽出を行った．

結　果

a) HBsAg 検査

HBsAg 測定者 6,648 例のうち，陽性者（HBV キャリア）は 126 例（1.9%）であった．そのうち血小板数< $15\times10^4/\mu L$，AST≤ALT，ALT≥31 IU/L を満たすものはそれぞれ 26 例，39 例，37 例であり，推定 B 型慢性肝炎は 66 例（1.0%）であった（Fig. 1a）．27 診療科のうち測定数の多い上位 10 科は，整形外科 870 例，眼科 727 例，産婦人科 531 例，一般・消化器外科 509 例，循環器内科 410 例，救急科 344 例，消化器内科 291 例，総合診療科 285 例，泌尿器科 270 例，皮膚科 252 例であった（Fig. 2a）．陽性者は，一般・消化器外科 20 例，総合診療科 16 例，整形外科 12 例，産婦人科 10 例，循環器内科 10 例，眼科 9 例，救急科 6 例，泌尿器科 6 例，消化器内科 5 例，腎臓内科 5 例の順で多く，これら上位 10 科のうち肝臓内科へのコンサルト率は内科系診療科で比較的高い傾向がみられ，整形外科や眼科は低率であった（Fig. 2b）．陽性率では総合診療科 5.6%，腎臓内科 4.0%，呼吸器内科 4.0%，一般・消化器外科 3.9%，循環器内科 2.4%，泌尿器科 2.2%，リウマチ・膠原病内科 2.2%，産婦人科 1.9%，救急科 1.7%，消化器内科 1.7% の順で高率であった．

Table 1 Characteristics of HBV carriers according to hepatologist consultation (n＝126)

	with consultation n＝26	without consultation n＝100	*P*-value
Age (years)	50 (19-78)	64 (16-91)	0.003
Males/females	13/13	43/57	0.658
Department: Internal Medicine/others	17/9	34/66	0.006
Platelet (×10⁴/μL)	20.2 (3.2-36.4)	20.1 (2.9-66.5)	0.545
AST (IU/L)	25 (12-450)	24 (9-558)	0.598
ALT (IU/L)	22 (9-93)	19 (5-189)	0.657
AST/ALT ratio	1.26 (0.57-4.84)	1.23 (0.40-5.58)	0.638
γ-GTP (IU/L)	17 (7-998)†	22 (8-1,590)‡	0.737
HBsAg (IU/mL)	1698 (0.07-21,792)	188 (0.07-106,400)	0.190
HBeAg positive/negative	2/17	7/61	1.000
with/without hepatitis	13/13	53/47	0.828

Values are median (range) or number of patients. †n＝25, ‡n＝85.

Table 2 Univariate and multivariate analysis of factors associated with hepatologist consultation for HBV carriers (n＝126)

Factors	Univariate analysis		Multivariate analysis	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Age	0.963 (0.937-0.989)	0.006	0.965 (0.938-0.992)	0.012
Sex: male	1.326 (0.558-3.148)	0.523		
Department: except Internal Medicine	0.273 (0.110-0.676)	0.005	0.294 (0.115-0.748)	0.010
Platelet (×10⁴/μL)	1.007 (0.958-1.058)	0.784		
AST (IU/L)	1.003 (0.997-1.008)	0.370		
ALT (IU/L)	1.003 (0.988-1.019)	0.688		
γ-GTP (IU/L)†	1.000 (0.998-1.002)	0.806		
HBsAg (IU/mL)	1.000 (1.000-1.000)	0.525		
HBeAg‡: positive	1.025 (0.195-5.397)	0.977		

†n＝110, ‡n＝87.

HBV キャリア 126 例のうち介入群は 26 例（21％），非介入群は 100 例（79％）であった（Fig. 1a）．2 群間の比較では，非介入群は有意に高齢であり，非内科系診療科が有意に多かった（Table 1）．また，コンサルトに寄与する因子の多変量解析でも，年齢（odds ratio [OR]：0.965，95% confidence interval [CI]：0.938–0.992, $p＝0.012$）と診療科（非内科系診療科, OR：0.294, 95％CI：0.115–0.748，$p＝0.010$）が関与していた（Table 2）．推定 B 型慢性肝炎 66 例のうちコンサルトに寄与する因子の単変量解析では，診療科（非内科系診療科，OR：0.269，95％CI：0.073–0.991，$p＝0.048$）が唯一関与しており，多変量解析では診療科（非内科系診療科，OR：0.270，95％CI：0.072–1.017，$p＝0.053$）で傾向を認めた（Table 3）．肝炎のない HBV キャリア 60 例のうちコンサルトに寄与する因子の単変量解析では，年齢（OR：0.958，95％CI：0.923–0.993，$p＝0.02$）と診療科（非内科系診療科, OR：0.265, 95％CI：0.074–0.954, $p＝0.042$）が関与しており，多変量解析では年齢（OR：961，95％CI：0.926–0.998，$p＝0.039$）が唯一関与していた．

非介入群の診療内容調査では，50 例は HBV について全く記載がなく，9 例は既往歴に記載があるのみでプロブレムリストには挙げられておらず，30 例はプロブレムリストに挙げられているものの診療方針が記載されていなかった（Table 4）．

Table 3 Univariate and multivariate analysis of factors associated with hepatologist consultation for probable chronic hepatitis B individuals (n = 66)

Factors	Univariate analysis		Multivariate analysis	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Age	0.969 (0.930-1.009)	0.125	0.969 (0.929-1.010)	0.140
Sex: male	1.227 (0.354-4.250)	0.747		
Department: except Internal Medicine	0.269 (0.073-0.991)	0.048	0.270 (0.072-1.017)	0.053
Platelet ($\times 10^4/\mu L$)	0.988 (0.927-1.054)	0.724		
AST (IU/L)	1.003 (0.997-1.009)	0.292		
ALT (IU/L)	1.006 (0.989-1.024)	0.482		
γ-GTP (IU/L)†	1.000 (0.998-1.002)	0.785		
HBsAg (IU/mL)	1.000 (1.000-1.000)	0.491		
HBeAg‡: positive	1.667 (0.151-18.455)	0.677		

†n = 60, ‡n = 46.

Table 4 Situation of medical practice for HBV carriers without hepatologist consultation (n = 100)

Descriptions regarding HBV on electronic medical record	n (%)
nothing	50 (50)
written in the past history, but not listed on the problem lists	9 (9)
listed on the problem lists, but not mentioned in the plan for medical practice	30 (30)
listed on the problem lists and mentioned in the plan for medical practice	11 (11)

b) Anti-HCV 検査

Anti-HCV 測定者 6,612 例のうち，陽性者は 487 例 (7.4%)，そのうち低力価 118 例，中力価 114 例，高力価 255 例であり，推定 HCV キャリアは 369 例 (5.6%) であった．そのうち血小板数$<15\times10^4/\mu L$, AST≦ALT, ALT≧31 IU/L を満たすものはそれぞれ 151 例，97 例，139 例であり，推定 C 型慢性肝炎は 244 例 (3.7%) であった (Fig. 1b)．27 診療科のうち測定数の多い上位 10 科は，整形外科 875 例，眼科 728 例，産婦人科 531 例，一般・消化器外科 519 例，循環器内科 402 例，救急科 334 例，消化器内科 286 例，総合診療科 277 例，泌尿器科 268 例，歯科口腔外科 263 例であった (Fig. 3a)．推定 HCV キャリア数は，整形外科 52 例，一般・消化器外科 50 例，眼科 47 例，消化器内科 25 例，循環器内科 25 例，救急科 22 例，総合診療科 15 例，腎臓内科 13 例，皮膚科 12 例，歯科口腔外科 12 例の順で多く，これら上位 10 科のうち肝臓内科へのコンサルト率は消化器内科，総合診療科，一般・消化器外科，救急科で比較的高い傾向がみられたものの 3 割前後に留まっていた (Fig. 3b)．陽性率では，腎臓内科 10.7%，一般・消化器外科 9.6%，消化器内科 8.7%，心臓血管外科 7.2%，救急科 6.6%，眼科 6.5%，呼吸器内科 6.3%，循環器内科 6.2%，脳神経外科 6.0%，整形外科 5.9% の順で高率であった．

推定 HCV キャリア 369 例のうち介入群は 67 例 (18%)，非介入群は 302 例 (82%) であった (Fig. 1b)．両群間の比較では，非介入群では血小板数が有意に多く，AST 値，ALT 値，γ-GTP 値，Anti-HCV 抗体価が有意に低値であった (Table 5)．また，コンサルトに寄与する因子の多変量解析では，ALT 値 (OR：1.014，95%CI：1.004–1.024，$p=0.007$) と Anti-HCV (OR：1.196，95% CI：1.073–1.334，$p=0.001$) が関与していた (Table 6)．推定 C 型慢性肝炎 244 例のうちコンサルトに寄与する因子の多変量解析では，血小板数 (OR：0.897，95%CI：0.831–0.968，$p=0.005$)，ALT 値 (OR：1.020，95%CI：1.008–1.033，$p=0.001$)，Anti-HCV (OR：1.283，95% CI：1.085–1.518，$p=0.004$) が関与していた (Table 7)．肝炎のない推定 HCV キャリア 125 例のうちコンサルト

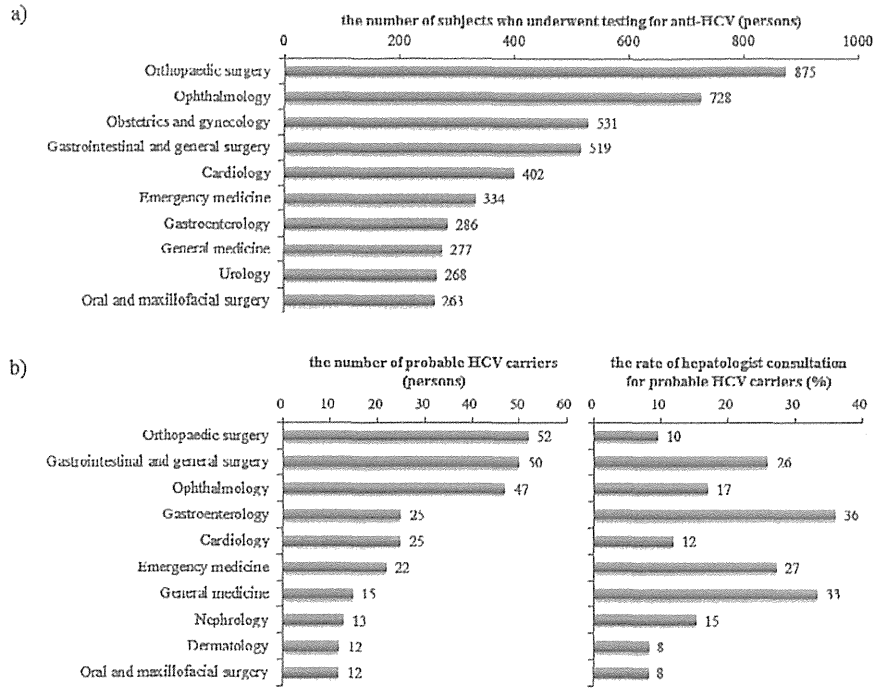

Fig. 3　a) The number of subjects who underwent anti-HCV screening test in the top 10 non-hepatology departments. b) The number of probable HCV carriers in the top 10 non-hepatology departments, and the rate of hepatologist consultation.

Table 5　Characteristics of probable HCV carriers according to hepatologist consultation (n＝369)

	with consultation n＝67	without consultation n＝302	*P*-value
Age (years)	69 (33-85)	71 (0-93)	0.113
Males/females	38/29	156/146	0.500
Department: Internal Medicine/others	25/84	42/218	0.139
Platelet ($\times 10^4/\mu L$)	13.9 (5.5-38.8)	16.7 (2.7-62.6)	0.014
AST (IU/L)	38 (13-116)	29 (10-822)	0.017
ALT (IU/L)	31 (5-162)	24 (4-230)	0.024
AST/ALT ratio	1.21 (0.41-3.00)	1.31 (0.34-7.76)	0.266
γ-GTP (IU/L)	30 (9-190)†	23 (7-564)‡	0.042
Anti-HCV (S/CO)	14.2 (5.6-18.8)	13.4 (4.0-18.5)	＜0.001
with/without hepatitis	46/21	198/104	0.671

Values are median (range) or number of patients. †n＝59, ‡n＝257.

に寄与する因子の多変量解析では，Anti-HCV（OR：1.200，95％CI：1.019-1.413，$p=0.029$）が唯一関与していた．

非介入群の診療内容調査では，150例（50％）はHCVについて全く記載がなく，63例（21％）は既往歴に記載があるのみでプロブレムリストには挙げられておらず，79例（26％）はプロブレムリストに挙げられているものの診療方針が記載されていなかった（Table 8）．

Table 6 Univariate and multivariate analysis of factors associated with hepatologist consultation for probable HCV carriers (n = 369)

Factors	Univariate analysis		Multivariate analysis	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Age	0.989 (0.969-1.009)	0.265		
Sex: male	1.226 (0.719-2.091)	0.453		
Department: except internal medicine	0.647 (0.371-1.128)	0.125	0.576 (0.320-1.034)	0.065
Platelet ($\times 10^4/\mu L$)	0.966 (0.927-1.006)	0.094	0.986 (0.948-1.026)	0.487
AST (IU/L)	1.002 (0.997-1.007)	0.382		
ALT (IU/L)	1.017 (1.007-1.027)	<0.001	1.014 (1.004-1.024)	0.007
γ-GTP (IU/L)†	1.001 (0.997-1.005)	0.744		
Anti-HCV (S/CO)	1.219 (1.096-1.355)	<0.001	1.196 (1.073-1.334)	0.001

† n = 316.

Table 7 Univariate and multivariate analysis of factors associated with hepatologist consultation for probable chronic hepatitis C individuals (n = 244)

Factors	Univariate analysis		Multivariate analysis	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Age	0.990 (0.965-1.015)	0.432		
Sex: male	1.206 (0.622-2.338)	0.580		
Department: except Internal Medicine	0.724 (0.370-1.418)	0.346		
Platelet ($\times 10^4/\mu L$)	0.902 (0.839-0.969)	0.005	0.897 (0.831-0.968)	0.005
AST (IU/L)	1.002 (0.997-1.007)	0.357		
ALT (IU/L)	1.022 (1.010-1.034)	<0.001	1.020 (1.008-1.033)	0.001
γ-GTP (IU/L)†	1.000 (0.996-1.005)	0.877		
Anti-HCV (S/CO)	1.290 (1.108-1.503)	0.001	1.283 (1.085-1.518)	0.004

† n = 211.

Table 8 Situation of medical practice for probable HCV carriers without hepatologist consultation (n = 302)

Descriptions regarding HCV on electronic medical record	n (%)
nothing	150 (50)
written in the past history, but not listed on the problem lists	63 (21)
listed on the problem lists, but not mentioned in the plan for medical practice	79 (26)
listed on the problem lists and mentioned in the plan for medical practice	10 (3)

考　察

今回我々が行った院内調査では，HBsAg陽性率1.9%，推定HCVキャリア率5.6%であり，平成18年度の全国調査における結果(HBsAg陽性率：全国1.0%，佐賀県1.8%；推定HCVキャリア率：全国0.8%，佐賀県2.7%)[6]に比して，高率であった．特に，C型肝炎が高率だが，これは大学病院受診者に高齢者が多いことに起因していると推測される．実際，推定HCVキャリアの年齢中央値は70歳であるが，今回の結果は佐賀県の最近10年間(2001～2010年)の70歳代におけるHCVキャリア率(5.2%)とほぼ一致する(未発表データ)．このように，院内で多くの肝炎ウイルスキャリアが判明しているに

もかかわらず，HBVキャリアの79%および推定HCVキャリアの82%で肝臓内科との連携がなく，そのうち，前者の89%および後者の97%において肝炎に関する診療方針も記載されていない状況であった．但し，カルテに記載のない症例においても，患者へ結果説明はなされている症例が存在すると推測され，今回の後ろ向き研究では結果の開示率は不明である．今後，スクリーニングで判明した陽性者が適切な治療まで結びついているかの追跡調査が必要と考えられる．

科別の測定数では，両検査とも整形外科，眼科，産婦人科，一般・消化器外科，循環器内科の順で多く，これらはほとんどが術前スクリーニング検査と推測される．HBVキャリアの肝臓内科へのコンサルトに関与する因子として，年齢と非内科系診療科が負の因子として抽出され，推定B型慢性肝炎例では，非内科系診療科のみが負の傾向を認めた．HBVキャリアの多い10診療科をみても，非内科系診療科はコンサルト率が低く，特に整形外科や眼科で低率であり，これらの診療科への働きかけが重要と考えられる．B型肝炎は複雑な病態を示すため[12]，HBsAg陽性（HBVキャリア）であれば肝臓専門医へのコンサルトが必須である．また，B型肝炎は家族内感染が多く[12]，さらに多くのHBVキャリアの掘り起しに繋がることも重要な点である．推定HCVキャリア全体ではコンサルトに関与する因子としてALT値とHCV抗体価が独立した因子であり，非内科系診療科も負の傾向を認めた．推定C型慢性肝炎例では，肝炎の活動性や線維化が軽度な症例ではコンサルトがされにくい状況が明らかとなった．C型肝炎は，ほとんどの症例で徐々に線維化の進展を来し，それに伴い肝発癌率が上昇していくため[12]，今回C型慢性肝炎と推定された症例では，肝臓専門医の介入は必須である．また，ALT持続正常例においても緩徐ではあるが肝線維化は進行するため[13]，慢性肝炎の可能性が低いとみなされたHCVキャリア症例の中にも，抗ウイルス治療を要する症例が存在する可能性は十分にあり，全科へ積極的な肝臓内科へのコンサルトを啓発していくことが重要である．

Anti-HCV感染率は腎臓内科で最も高いが，これは透析患者の高い罹患率やHCVの肝外病変としての腎疾患を反映しているものと推測される．透析患者においてもHCV感染が生命予後を悪化させることが明らかにされており，抗ウイルス療法を行うことが推奨されている[14]．また，透析患者では血清トランスアミナーゼ値が低値であるため[14]，治療を行うべき症例が見落とされている可能性もあり，肝臓専門医の判断が必要である．一般・消化器外科ではHBsAg，Anti-HCVともに陽性者が多いが，肝癌に対する手術が行われている影響と考えられ，これらの患者は肝疾患専門のかかりつけ医を受診している可能性が高い．一方で，anti-HCV陽性者の多い眼科や整形外科では肝疾患として医療機関を受診している可能性が低いことが危惧される．眼科における感染症スクリーニングの報告は散見されるが，HBsAg陽性率0.5～1.4%，Anti-HCV陽性率4.3～5.8%と，我々の結果と同様にHCVにおいて高率である[15)～18)]．しかし，いずれの報告も医療者側の感染予防対策に論点が限られており，肝炎ウイルスキャリアの肝炎診療についての言及は皆無であった．特に整形外科など非内科系診療科では同様の傾向にあるものと推測され，肝炎ウイルス検査を行うことの多い診療科に対して，検査陽性時の対応を明確に示しておくことが重要であると考えられる．

これまで，肝炎対策の問題点として，検診における肝炎ウイルス検査受検率や要精密者の医療機関受診率の低さ，さらには一般医療機関での肝疾患診療の不備などが指摘されてきた[8]．ところが，大学病院のような高次医療機関においても，多くの肝炎ウイルスキャリアが肝疾患診療に結びついていないという事実が，今回明らかとなった．これまで当院で非肝臓内科において専門的な情報提供や精査の機会を逸した肝炎ウイルス陽性患者については，倫理委員会の承認の元，可能な限り遡及調査とフォローアップを行うべく，まず平成20年度以降の現在の電子カルテからの抽出作業に着手している．さらに，厚生労働省が平成23年度の施策として，肝炎治療促進の環境整備のために「地域肝炎治療コーディネーター養成事業」を挙げ，佐賀県においても，135名のコーディネーターを養成し，当院でも活躍しているところであり，現在の診療データでの評価が可能な症例や通院を継続している症例については，可能な限り主診療科と主治医の協力の元，肝炎コーディネーターによる肝疾患の受療の調査および情報提供を行うことを計画している．しかし，大学病院の特性から追跡困難な症例もあり，その場合は，可能な限り，紹介先の医療機関への情報提供を行うべきであろうと考える．またそれらの症例の中では，測定時と比べて肝疾患の進展を来している症例も少なからずは存在することが想定され，それらの症例へは個々に最善のアプローチと対策が強く望まれる．プロスペクティブな体制としては，現在，外来診療委員会から各科への肝

炎ウイルス陽性者へのコンサルテーション推進の周知がなされ，肝炎コーディネーターへの介入依頼が見られる．さらに，次期電子カルテシステムではウイルス陽性者が発生した際に，電子カルテ上で自動的に主治医に通知し，肝臓専門医へのコンサルテーションを促すシステムと主治医の依頼によって肝炎コーディネーターが対象者に対する個別指導を行うための指導ツールの開発，運用を検討している．肝炎ウイルスキャリア率が高く，かつ肝臓専門医を擁する医療機関において，肝炎ウイルスキャリアに対する適切な肝炎診療を行うための院内連携システムを構築することは，肝炎対策において非常に重要な位置を占めるものと考えられる．

結　語

大学病院の非肝臓内科において，多くのHBsAgおよびAnti-HCVが測定されていたが，その陽性率が非常に高いにもかかわらず，介入を要すると推測される肝炎ウイルスキャリア症例の約8割が肝臓内科に紹介されていないという事実が明らかとなった．非肝臓内科におけるHBsAgおよびAnti-HCV陽性者を適切な肝疾患診療に結びつける院内連携システムの構築が，今後，早急に必要である．

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本論文内容に関連する著者の利益相反：なし

Current management practices for HBs antigen or anti-HCV antibody positive individuals in non-hepatology departments at a university hospital

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Little is known about the current medical management practices relating to hepatitis virus carriers in non-hepatology departments. The aim of this study was to clarify the existing management of viral hepatitis in non-hepatology departments at a university hospital. Subjects who underwent screening tests for HBsAg (n＝6,648) and anti-HCV (n＝6,612) at 27 non-hepatology departments between January 2010 and December 2010 were analyzed. The number of HBsAg-positive (HBV carrier), probable chronic hepatitis B, anti-HCV-positive, probable HCV carrier, and probable chronic hepatitis C were 126 (1.9%), 66 (1.0%), 487 (7.4%), 369 (5.6%), and 244 (3.7%), respectively. In spite of high infection rates, 79% of HBV carriers and 82% of probable HCV carriers were not referred to a hepatologist. In 89% of the former and 97% of the latter, a medical plan for viral hepatitis was not described in the electronic medical record. A system to manage hepatitis virus carriers should be established immediately in medical institutions that have hepatologists.

Key words: hepatitis virus　HBs antigen　anti-HCV antibody　screening　management of hepatitis virus carriers

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ORIGINAL ARTICLE – HEPATOBILIARY TUMORS

The Significance of Classifying Microvascular Invasion in Patients with Hepatocellular Carcinoma

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ABSTRACT

Background. Microvascular invasion (MVI) has been recognized as a risk factor for outcome following curative resection in hepatocellular carcinoma (HCC). Because MVI can range from few to many invaded vessels, we evaluated the significance of MVI classification in this study.

Methods. Between January 1995 and December 2010, 207 consecutive patients who underwent curative resection for HCC within Milan criteria were included in this retrospective study. Patients were classified into mild and severe MVI groups based on the number of vessels invaded. This study evaluated whether MVI classification can help to predict recurrence and survival after curative resection.

Results. Of the total 207 patients, 103 (50 %) patients had no detectable MVI, whereas 59 (28 %) had mild MVI, and 45 (22 %) had severe MVI. Recurrence-free survival rates at 2 years for patients without MVI, with mild MVI, and severe MVI were 75.9, 47.2, and 32.7 %, respectively. Patients with severe MVI experienced a high frequency of fatal recurrence, such as multiple tumors, macroscopic vascular invasion, and extrahepatic metastasis after curative resection. Multivariate analysis revealed age, number of tumors, mild MVI, and severe MVI as independent predictors of recurrence-free survival. Disease-specific survival rates at 5 years for patients without MVI, with mild MVI, and severe MVI were 91.5, 70.4, and 51.4, respectively. Multivariate analysis also revealed cirrhosis, tumor size, mild MVI, and severe MVI as independent predictors of disease-specific survival.

Conclusions. We demonstrated that MVI classification can stratify HCC patients by different patterns of recurrence and risk of survival after curative resection.

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Hepatocellular carcinoma (HCC) is one of the most common malignancies in the world. Recent advances in imaging procedures and surveillance programs for high-risk patients have led to increased detection of early-stage HCC, resulting in an increase in identification of patients in whom curative resection is possible.[1,2] However, the long-term survival of HCC patients remains unsatisfactory due to the high frequency of intra- and extrahepatic recurrences.[3,4] Vascular invasion (VI) has been recognized as a risk factor leading to early recurrence of HCC.[5–8] Moreover, the fatal recurrence in HCC patients with VI limits additional attempts at various curative therapies, such as liver resection and radiofrequency ablation (RFA), thereby contributing to poor survival.[9,10]

VI is generally classified using either macroscopic or microscopic findings. Macroscopic VI, such as a tumor thrombus in the major portal vein, is known to be a crucial risk factor for survival after liver resection or transplantation in HCC patients and is detectable by various imaging procedures.[11–14] Therefore, the presence of macroscopic VI is usually evaluated before treatment and is an important parameter that is included in the TNM, CLIP, JIS, and BCLC scoring systems and is used to determine treatment

strategies in HCC patients.[10,15,16] In contrast, microscopic vascular invasion (MVI) is difficult to detect before initiation of HCC treatment, even if sophisticated imaging procedures are conducted during patient evaluation. Recently, many studies have reported that the presence of MVI is closely associated with outcome following liver resection or transplantation in HCC patients.[17–21] Our previous study also showed that MVI was an important strong risk factor for recurrence and survival following resection of HCC within the Milan criteria.[22] Therefore, MVI is as important as macroscopic VI and should be evaluated as a risk factor of patients with HCC. Moreover, the current definition of MVI encompasses a wide range of tumor invasion, from one to many microscopic vessels that are contiguous with the tumor. Thus, patients with MVI have been suggested to have a wide range of outcomes after resection. Consequently, the purpose of this study was to evaluate whether the classification of MVI based on number of vessels invaded affects tumor recurrence and survival after resection of HCC.

METHODS

Patients

Between January 1995 and December 2010, 256 patients underwent liver resection at the Kurume University School of Medicine and were diagnosed with HCC by histological findings. The following patients were excluded: (1) patients whose disease did not fulfill the Milan criteria (a single tumor $\leq$5 cm or $\leq$3 tumors each $\leq$3 cm); (2) patients with macroscopic VI; (3) patients with extrahepatic metastasis; (4) patients who underwent noncurative liver resection; and (5) patients who were diagnosed with combined hepatocellular and cholangiocellular carcinoma by histological findings. Of the total 256 patients, 49 patients having one or more of the above criteria were excluded, and the remaining 207 patients were retrospectively enrolled in this study. Patients included 162 males (78 %) and 45 females, with a median age of 66 (range 16–83) years. Overall, 147 patients (71 %) were positive for hepatitis C virus (HCV) infection and 46 patients (22 %) were positive for hepatitis B virus (HBV) infection. Liver cirrhosis was present in 81 patients (39 %). The median tumor size was 25 (range 12–50) mm, and 160 patients (77 %) had a solitary tumor. Various surgical procedures were classified as major or minor resections according to Couinaud's segment classification. Major resections (segmentectomy, sectorectomy, and lobectomy or greater) were performed in 123 patients, whereas minor resections (all other types of resection, including partial hepatectomy and subsegmentectomy) were performed in 84 patients.

Follow-Up and Endpoint

After surgical resection, each patient was followed carefully. Serum biochemistries, alpha-fetoprotein (AFP) levels, and des-gamma-carboxy prothrombin (DCP) levels were measured, and ultrasonography was performed monthly. Contrast-enhanced dynamic computed tomography (CT) was performed every 3 months until 6 months posttreatment and every 6 months thereafter. Magnetic resonance imaging (MRI) was performed as a supplemental examination. Recurrence was diagnosed based on the combined findings of these examinations with appearances typical of HCC. The endpoint of this study was the date of recurrence, death, or last follow-up visit; the closing date was December 2011. The median duration of follow-up was 54.4 (range 9.5–177.8) months.

Histopathological Evaluation

The resected liver specimens were cut into serial 2–3-mm thick slices and fixed in 10 % formalin to facilitate careful gross and histopathological examinations. Each of the liver slices was embedded in paraffin, cut into 4-mm sections, and stained with hematoxylin and eosin. Tumors were examined for maximum tumor size, MVI, intrahepatic micrometastasis, capsular formation, and histologic grade. Noncancerous liver parenchyma was inspected for evidence of cirrhosis. Histological grade was based on the criteria of the Edmondson-Steiner classification and the Liver Cancer Study Group of Japan.[23,24] Intrahepatic micrometastasis was defined as a satellite micronodule in the surrounding liver tissue that is isolated from the main tumor. MVI was defined as microscopic tumor invasion identified in the portal vein and hepatic vein of the surrounding liver tissue that is contiguous with the tumor edge. Moreover, we hypothesize that the extent of MVI may affect tumor recurrence and survival after resection, because MVI encompasses a wide range of tumor invasion. Therefore, in this study, we devised a novel classification of MVI based on number of invaded vessels and divided the patients with MVI into two groups as follows: patients in the mild MVI group had one to five invaded vessels, whereas patients in the severe MVI group had more than five invaded vessels. The number of MVI was counted in each nodule. If patients had multiple tumors, the tumor with the most number of invaded vessels was selected for the classification of MVI. These histopathological evaluations of the resected specimens were retrospectively performed by one experienced pathologist (O.N.). Typical examples of the two MVI types are shown in Fig. 1.

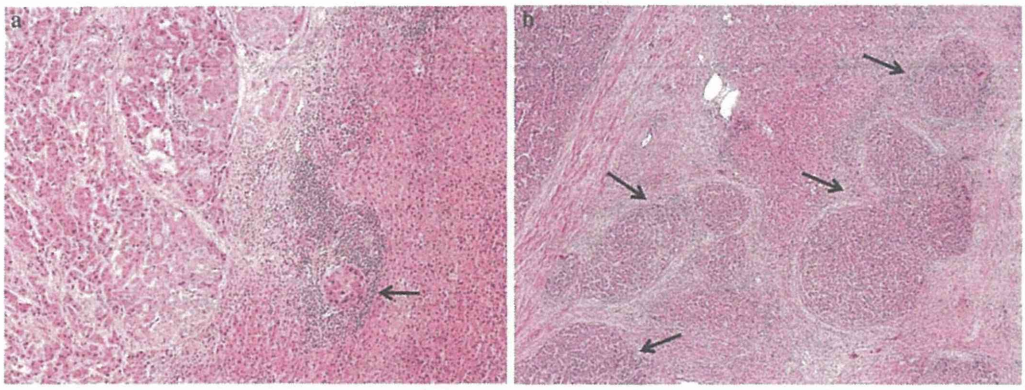

FIG. 1 Typical examples of the two microvascular invasion (MVI) types classified by number of vessels invasion. **a** mild MVI: peritumoral vessel invasion from one to five (hematoxylin–eosin, ×50). **b** severe MVI: peritumoral vessel invasion more than five (hematoxylin–eosin, ×20)

Statistical Analysis

Continuous variables were expressed as median (range). Comparison analysis among MVI grades was performed using the Chi square test for discrete variables and the Kruskal–Wallis test followed by Mann–Whitney *U* test with Bonferroni correction as a post hoc test for continuous variables. Recurrence-free survival and disease-specific survival were determined by Kaplan–Meier analysis, and differences between subgroups were compared with log-rank tests. A cause specific Cox proportional hazards model was used for univariate and multivariate analysis to identify separately any independent variables that were related to recurrence-free survival or disease-specific survival. The variables that were statistically significant by univariate analysis were included in a multivariate analysis. No interaction terms were considered, because the preanalysis showed the nonsignificance for the interaction. Data from these models were expressed as hazard ratio (HR) and 95 % confidence interval (95 % CI). All *P* values were two-tailed, and a level of <0.05 was considered to be statistically significant. Statistical analysis was performed by SPSS software version 20 (SPSS Inc., Chicago, IL, USA).

RESULTS

Comparison of Patient Characteristics Based on Grade of MVI and Predictors of Severe MVI

Clinicopathological characteristics of this study population stratified by grade of MVI are shown in Table 1. Of the total 207 patients, 103 (50 %) patients had no detectable MVI, whereas 59 (28 %) had mild MVI, and 45 (22 %) had severe MVI on pathologic examination. Patients with severe MVI had significantly higher elevated AFP and DCP levels compared with patients without MVI or with mild MVI. Patients with worse MVI grades had larger tumors and a significantly higher prevalence of HCC that was poorly differentiated and had intrahepatic micrometastasis. Other clinicopathological characteristics did not differ significantly among the three groups.

Recurrence-Free Survival and Predictive Factors

Factors associated with recurrence-free survival were evaluated by univariate and multivariate analyses. Univariate analysis showed that age >65 years, HCV infection, HBV infection, elevated DCP level, tumor size >20 mm, presence of multiple tumors, presence of MVI, and presence of intrahepatic metastasis were significant variables affecting recurrence-free survival (Table 2). By multivariate analysis, presence of MVI (mild MVI; hazard ratio [HR]: 1.93, 95 % confidence interval [CI]: 1.25–2.98, $P = 0.003$ and severe MVI; HR: 2.87, 95 % CI: 1.85–4.46, $P < 0.001$), age (>65 years; HR: 1.84, 95 % CI: 1.27–2.65, $P = 0.001$), and number of tumors (2–3; HR: 1.68, 95 % CI: 1.13–2.51, $P = 0.011$) were identified as independent predictors of recurrence-free survival (Table 3). Recurrence-free survival curves of patients stratified by grade of MVI are shown in Fig. 2a. The recurrence-free survival of patients with mild and severe MVI was significantly shorter than that of patients without MVI (no MVI vs. mild MVI, $P = 0.0001$; no MVI vs. severe MVI, $P < 0.0001$; mild MVI vs. severe MVI, $P = 0.1663$).

Pattern and Treatment of First Recurrence after Resection

During the follow-up period, tumor recurrence developed in 122 (54 %) patients, consisting of 48 (47 %) patients without MVI, 38 (64 %) patients with mild MVI, and 36 (80 %) patients with severe MVI. The majority of