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Original Article

Wisteria floribunda agglutinin positive human Mac-2-binding protein as a predictor of hepatocellular carcinoma development in chronic hepatitis C patients

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Aims: *Wisteria floribunda* agglutinin (WFA)-positive human Mac-2-binding protein (WFA$^+$-M2BP) is a new glycol marker related to liver fibrosis. The aim of the present study was to evaluate WFA$^+$-M2BP as a predictor of hepatocellular carcinoma (HCC) development in patients with chronic hepatitis C.

Methods: This case–control study included 14 patients with chronic hepatitis C who developed HCC and 52controls, matched for age, gender, and fibrosis stage. WFA$^+$-M2BP was measured at biopsy and follow-up. Time zero was set at the date of liver biopsy.

Results: WFA$^+$-M2BP increased stepwise with progression of liver fibrosis ($p < 0.001$). Cumulative incidence of HCC development was significantly higher in patients with WFA$^+$-M2BP $\geq$4.2 ($p < 0.001$) or in those with time-course changes in WFA$^+$-M2BP (ΔWFA$^+$-M2BP/year) $\geq$0.3 ($p = 0.03$). Multivariate analyses demonstrated that WFA$^+$-M2BP $\geq$4.2 [hazard ratio (HR): 4.1, 95% confidence interval (CI): 1.1–15, $p = 0.04$], ΔWFA$^+$-M2BP/year $\geq$0.3 (HR: 5.5, 95% CI: 1.5–19, $p = 0.008$), and AFP $\geq$10 ng/ml (HR: 4.7, 95% CI: 1.1–19, $p = 0.03$) were independent predictive factors of HCC development. Based on these data, we developed a simple scoring system to predict HCC development using these three factors. Using these scores, patients were classified into four groups; cumulative incidence of HCC development significantly increased with increasing scores ($p < 0.001$).

Conclusions: WFA$^+$-M2BP measurements and time-course changes in WFA$^+$-M2BP can be used to identify patients at high risk of HCC development. Real-time monitoring of WFA$^+$-M2BP can be a novel predictor of HCC development.

Key words: chronic hepatitis C, hepatocellular carcinoma, liver fibrosis, WFA$^+$-M2BP

INTRODUCTION

HEPATITIS C VIRUS (HCV) INFECTION is the major cause of chronic hepatitis, which progresses to hepatocellular carcinoma (HCC) in many patients.[1] Advanced stage liver fibrosis is associated with HCC development;[2] therefore, accurate staging of liver fibrosis is extremely important in clinical practice. Although liver biopsy is the gold standard to diagnose liver fibrosis,[3,4] this method may be inaccurate because of sampling errors and interobserver variations.[5,6]

In recent years, several alternative non invasive methods for evaluating liver fibrosis have emerged. It has been reported that liver fibrosis can be predicted by transient elastography,[7,8] acoustic radiation force impulse imaging[9] and real time tissue elastography[10,11] using ultrasonography. In addition, blood tests, such as the aspartate amino transferase (AST)/platelet ratio index (APRI)[12,13] and FIB 4 index,[14,15] have been reported to be useful in predicting liver fibrosis. Furthermore, these non invasive markers have been reported to be associated with HCC development and liver related mortality.[16–21]

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Glycans are referred to as the face of cells. Glycans mutate according to disease status, demonstrating their potential as biomarkers for chronic disease. In patients with chronic hepatitis, glycomic and glycoproteomic bio marker methods have also been reported to be useful in the diagnosis of liver fibrosis.[22,23]

Wisteria floribunda agglutinin (WFA) positive human Mac 2 binding protein (WFA^+ M2BP), a new glycol marker related to liver fibrosis, is obtained using sand wich immunoassay with WFA and anti M2BP antibody. This marker glycoprotein has demonstrated fibrosis related glyco alteration potential.[24] The significance of WFA^+ M2BP as a predictor of liver fibrosis in chronic HCV infection has been previously reported;[24,25] how ever, the relationship between WFA^+ M2BP and HCC remains unclear. The aim of this study was to evaluate WFA^+ M2BP as a predictor of HCC development.

METHODS

Patients

WE CONDUCTED A matched case control study to assess the relationship between WFA^+ M2BP and HCC development. Of patients with chronic HCV infection who underwent liver biopsy at Musashino Red Cross Hos pital (Tokyo, Japan) between 2002 and 2010 and did not achieve sustained virological response to interferon therapy, 14 who developed HCC were enrolled in this study. Fifty two patients who did not develop HCC served as matched controls on the basis of sex, age and histological fibrosis stage. WFA^+ M2BP was measured for all patients at biopsy and at more than 1 year follow up (mean interval, 2.6 ± 1.8 years). Exclusion criteria were as follows: (i) co infection with hepatitis B virus or HIV; (ii) history of autoimmune hepatitis or primary biliary cirrhosis; or (iii) history of HCC at study entry. Time zero was set at the date of liver biopsy. Written informed consent was obtained from each patient. The study protocol conformed to the ethical guidelines of the Declaration of Helsinki and was approved by the institutional ethics review committees.

Histological evaluation

Liver biopsy specimens were laparoscopically obtained using 13 G needles. If laparoscopy was contraindicated because of a history of upper abdominal surgery, percuta neous ultrasound guided liver biopsy was performed using 15 G needles. Specimens were fixed, paraffin embedded and stained with hematoxylin eosin and Masson trichrome. All liver biopsy samples were indepen dently evaluated by two senior pathologists who were blinded to the clinical data. Fibrosis staging was catego rized according to the METAVIR score: F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis with few septa; F3, numerous septa without cirrhosis; and F4, cir rhosis.[26] If staging was inconsistent between the two pa thologists, an appropriate stage was determined by means of a discussion between them.

HCC surveillance and diagnosis

Ultrasonography and blood tests (including tumor marker testing) were performed on all patients every 3 6 months for HCC surveillance. In cases of an increase in tumor marker level or abdominal ultrasonography suggestive of lesions suspicious for HCC, contrast enhanced computed tomography, magnetic resonance imaging or angiography was performed. HCC was diagnosed for tumors that showed vascular enhancement at an early phase with washout at a later phase. Tumor biopsy was used to diag nose tumors with non typical imaging results.

WFA^+-M2BP quantification using sandwich immunoassay with WFA and anti-M2BP antibody

The fibrosis specific glycosylated M2BP form was mea sured by sandwich immunoassay. Glycosylated M2BP was captured by WFA immobilized on magnetic beads. The bound product was assayed with an antihuman M2BP monoclonal antibody linked to alkaline phospha tase (ALP αM2BP). Assay manipulation was fully auto mated using a chemiluminescence enzyme immunoassay machine (HISCL 2000i; Sysmex, Kobe, Japan) and ac quired within 17 min.[24] All counts were standardized and converted to a cut off index designated as WFA^+ M2BP.[27]

WFA^+ M2BP was measured two times for all patients: at biopsy and follow up. Time course changes in WFA^+ M2BP (ΔWFA^+ M2BP/year) were calculated using the following formula:

$$\Delta WFA^+\text{-M2BP/year} = \frac{WFA^+ - \text{M2BP at follow up} - WFA^+\text{-M2BP at a liver biopsy}}{\text{interval between the two measurements (years)}}$$

Clinical and biological data

Patient age and sex were recorded. Serum samples were collected at liver biopsy and the following values were obtained through serum sample analyses: bilirubin, AST, alanine aminotransferase (ALT), platelet count and α fetoprotein (AFP). APRI and FIB 4 were calculated at liver biopsy, as previously reported.[12,14]

Statistical analyses

Categorical data were compared using the χ^2 test and Fisher's exact test. Distributions of continuous variables were analyzed using Student's *t* test or the Mann Whitney *U* test. Correlations between the WFA+ M2BP and histological fibrosis stage were analyzed using Spearman's rank correlation coefficients. $P < 0.05$ was considered statistically significant. Receiver operator curves (ROC) were constructed, and the area under the ROC (AUROC) was calculated. The cumulative incidence curve was determined by the Kaplan Meier method, and differences among groups were assessed using a log rank test. Factors associated with HCC risk were determined by the Cox proportional hazard model. Statistical analyses were performed using the Statistical Package for the Social Sciences software version 20.0 (SPSS, Chicago, IL, USA).

RESULTS

Patient characteristics

PATIENT CHARACTERISTICS AT biopsy are listed in Table 1. Age, sex, AST, ALT, bilirubin levels, platelet counts and histological fibrosis stage were not significantly different between patients with HCC development and patients without HCC development. The mean follow up period for all patients was 4.1 years.

Table 1 Patient characteristics

	Patients with HCC development ($n = 14$)	Patients without HCC development ($n = 52$)	*P*
Age (years)	65.2 ± 6.2	60.8 ± 9.6	0.1
Sex (male/female)	9/5	22/30	0.1
AST (IU/L)	69.1 ± 43	50.4 ± 31	0.07
ALT (IU/L)	74.5 ± 61	52.5 ± 34	0.08
Bilirubin (mg/dL)	0.74 ± 0.3	0.72 ± 0.3	0.8
Platelet counts ($\times 10^9$/L)	125 ± 38	144 ± 51	0.2
Fibrosis stage (1/2/3/4)	3/3/5/3	15/18/15/4	0.4
AFP (ng/mL)	28.2 ± 36	11.3 ± 18	0.02
WFA+ M2BP (COI)	4.70 ± 4.0	2.42 ± 2.2	0.007

AFP, α fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; COI, cut off index; HCC, hepatocellular carcinoma.

Relationship between histological findings and WFA+-M2BP

The relationship between histological findings and WFA+ M2BP was evaluated. Figure 1 shows median WFA+ M2BP compared with the METAVIR fibrosis stage. Median WFA+ M2BP increased stepwise with progression of liver fibrosis; levels for the F1, F2, F3 and F4 stages were 0.81, 1.82, 2.31 and 7.50, respectively ($P < 0.001$).

Prediction of HCC development by WFA+-M2BP and time-course changes in WFA+-M2BP

The AUROC of WFA+ M2BP for prediction of HCC development within 5 years was 0.768, and a WFA+ M2BP level of 4.2 was selected as the optimal cut off value. The cumulative incidence of HCC development was significantly higher in patients with WFA+ M2BP of 4.2 or more than those with WFA+ M2BP less than 4.2 ($P < 0.001$, Fig. 2A). Similarly, AUROC of ΔWFA+ M2BP/year for prediction of HCC development within 5 years was 0.607, and the optimal ΔWFA+ M2BP/year cut off value of 0.3 was selected. The cumulative incidence of HCC development was significantly higher in patients with ΔWFA+ M2BP/year of 0.3 or more than those with ΔWFA+ M2BP/year of less than 0.3 ($P = 0.03$, Fig. 2b). AUROC for APRI, FIB 4, platelet count and AFP was 0.708, 0.736, 0.674 and 0.822, respectively (Fig. 3). Besides AFP, WFA+ M2BP was more accurate for predicting HCC development than fibrosis stage and other fibrosis markers.

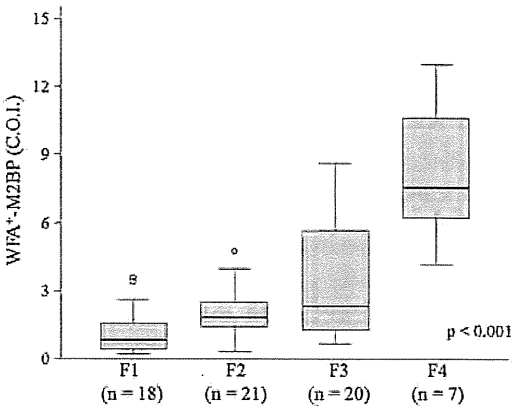

Figure 1 Correlation between WFA+ M2BP and fibrosis stage. Box plot of WFA+ M2BP is shown for each fibrosis stage. The box plot represents the 25th to 75th percentiles, giving the interquartile range. The line through the box indicates the median value, and an error bar indicates minimum and maximum non extreme values.

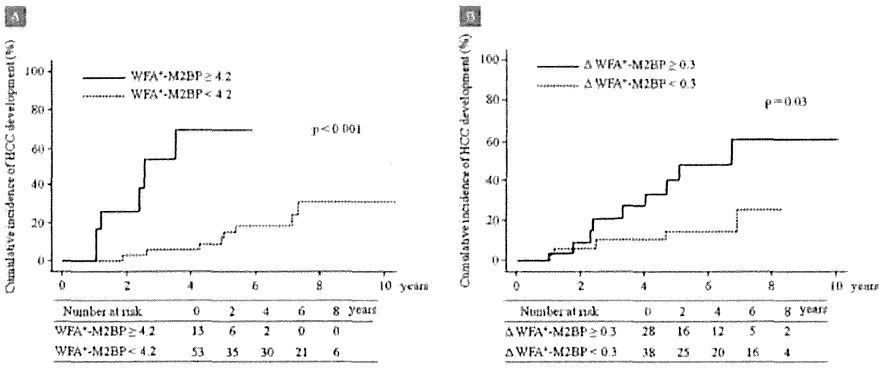

Figure 2 Cumulative incidence of hepatocellular carcinoma (HCC) development. Patients were categorized into two groups according to (a) WFA$^+$ M2BP and (b) time course change in WFA$^+$ M2BP.

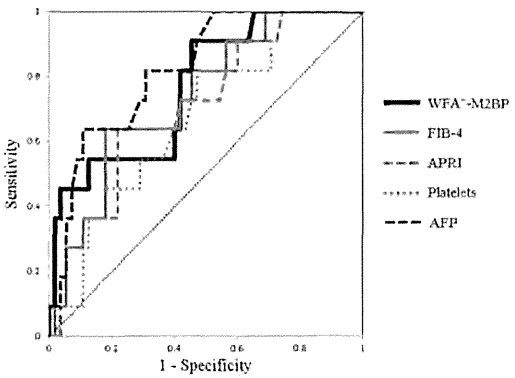

Figure 3 Receiver operator curves of WFA+ M2BP, fibrosis markers and α fetoprotein (AFP). APRI, aspartate aminotransfer ase to platelet ratio index.

Factors associated with HCC development

Univariate analysis revealed factors that increase the HR for HCC development (Table 2). High WFA$^+$ M2BP levels and high ΔWFA$^+$ M2BP/year levels were risk factors for HCC development. Compared with patients with WFA$^+$ M2BP of less than 4.2, HR for those with WFA$^+$ M2BP of 4.2 or more was 8.2 (95% confidence interval [CI], 2.6 26; $P < 0.001$). Similarly, patients with ΔWFA$^+$ M2BP/year of 0.3 or more had a HR of 3.1 compared with those with ΔWFA$^+$ M2BP/year of less than 0.3 (95% CI, 1.1 9.3; $P = 0.04$). Multivariate analyses demonstrated that WFA$^+$ M2BP, ΔWFA$^+$ M2BP/year and AFP levels were indepen dent predictive factors for HCC development (Table 2). HR for HCC development with WFA$^+$ M2BP of 4.2 or more, ΔWFA$^+$ M2BP/year of 0.3 or more and AFP of 10 ng/mL or more were 4.1 (95% CI, 1.1 15; $P = 0.04$), 5.5 (95% CI, 1.5 19; $P = 0.008$) and 4.7 (95% CI, 1.1 19; $P = 0.03$), respectively. We developed a scoring system based on these three factors. WFA$^+$ M2BP of 4.2 or more, ΔWFA$^+$ M2BP/year of 0.3 or more and AFP of 10 ng/mL or more each contributed 1 point to the score. WFA$^+$ M2BP of less than 4.2, ΔWFA$^+$ M2BP/year of less than 0.3 and AFP of less than 10 ng/mL each contributed 0 points to the score. Using this scoring system, patients were classi fied into four groups according to the total score of 0, 1, 2 or 3. Cumulative incidence of HCC development signifi cantly increased as the score increased ($P < 0.001$, Fig. 4).

DISCUSSION

RECENTLY, SEVERAL NON INVASIVE methods to evaluate liver fibrosis have been developed. The WFA$^+$ M2BP glycol marker test using sandwich immuno assay with WFA and anti M2BP antibody has demon strated utility as a liver fibrosis marker.[24,25] However, the relationship between WFA$^+$ M2BP and HCC develop ment remains unknown. The aim of this study was to de termine whether WFA$^+$ M2BP could be used to predict HCC development.

The important findings in this study were that WFA$^+$ M2BP and time course changes in WFA$^+$ M2BP indepen dently predicted HCC development. It is widely known that advanced liver fibrosis is associated with HCC development.[2] Non invasive markers of liver fibrosis are reported to be associated with HCC development and liver related mortality.[16–21] The correlation of liver fibrosis and WFA$^+$ M2BP was demonstrated in the present study. Patients with a high level of WFA$^+$ M2BP have been sug gested to have advanced liver fibrosis. Hence, we demon strated that cumulative incidence of HCC development was higher in patients with high WFA$^+$ M2BP levels than those with low WFA$^+$ M2BP levels. WFA$^+$ M2BP proved

Table 2 Factors associated with HCC development

		Univariate		Multivariate	
		HR (95% CI)	*P*	HR (95% CI)	*P*
Age (every 10 years)		2.0 (0.8 5.0)	0.1		
Sex (male/female)	Male	1			
	Female	0.4 (0.1 1.2)	0.1		
AST (IU/L)	<40	1			
	≥40	2.5 (0.6 11)	0 2		
ALT (IU/L)	<40	1			
	≥40	1.3 (0.4 3.7)	0.6		
Bilirubin (mg/dL)		2.8 (0.3 24)	0 3		
Platelet counts (×10^9/L)	≥150	1			
	<150	2.4 (0.6 8.4)	0 2		
Fibrosis stage	F1/2	1		1	
	F3/4	3.9 (1.3 11)	0.01	1.8 (0.5 6.3)	0.3
AFP (ng/mL)	<10	1		1	
	≥10	5.8 (1.8 18)	0.003	4.7 (1.1 19)	0.03
WFA+ M2BP (COI)	<4.2	1		1	
	≥4.2	8.2 (2.6 26)	<0.001	4.1 (1.1 15)	0.04
ΔWFA+ M2BP/year	<0.3	1		1	
	≥0.3	3.1 (1.1 9.3)	0.04	5.5 (1.5 19)	0.008

AFP, α fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CI, confidence interval; COI, cut off index; HCC, hepato cellular carcinoma; HR, hazard ratio.

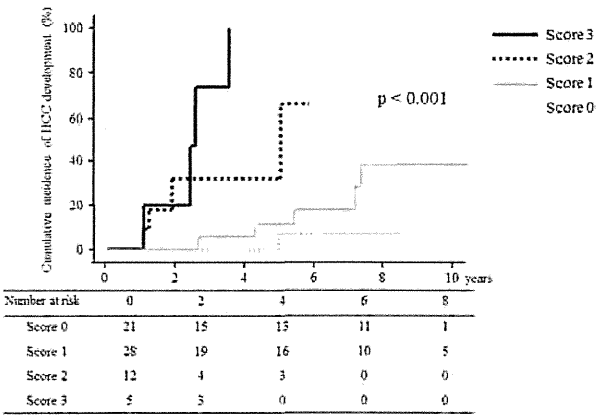

Figure 4 Association between the risk score and cumulative inci dence of hepatocellular carcinoma (HCC) development. WFA+ M2BP of 4.2 or more, ΔWFA+ M2BP/year of 0.3 or more and AFP of 10 ng/mL or more each contributed 1 point to the score. WFA+ M2BP of less than 4.2, ΔWFA+ M2BP/year of less than 0.3 and α fetoprotein (AFP) of less than 10 ng/mL each contributed 0 points to the score. Patients were classified into four groups according to the total score of 0, 1, 2 or 3.

to be a significant predictive factor for HCC development. In a recent study, the significance of WFA+ M2BP for pre diction of HCC was demonstrated in a large cohort study, further confirming the clinical impact of WFA+ M2BP.[28]

A new finding of our study was that time course changes in WFA+ M2BP were associated with HCC development. An advantage of WFA+ M2BP testing over liver biopsy is that its non invasiveness is suitable for repeated measure ment. Liver biopsy is problematic to repeat to assess time course changes because of its invasiveness.[29] WFA+ M2BP quantification can be used for real time monitoring of liver disease, based on our finding that time course changes were associated with HCC development. Further more, WFA+ M2BP and time course changes in WFA+ M2BP were independent predictors of HCC development, and patients at high risk of HCC development could be identified using a combination of these factors. Therefore, single point WFA+ M2BP assessment plus time course changes in WFA+ M2BP are more useful to predict HCC development than a single point liver biopsy.

WFA+ M2BP has some advantages over other serum fi brosis markers and elastography. Although APRI and FIB 4 serum fibrosis markers have demonstrated utility in predicting HCC development,[19–21] they are calculated using AST, ALT, platelet count and age. Hence, APRI and FIB 4 may not be appropriate in cases of advanced age, fatty liver or interferon therapy.[30] Furthermore, diagnostic accuracy of APRI and FIB 4 for HCC development was in ferior to WFA+ M2BP in this study.

Liver elastography using ultrasonography has utility in predicting HCC development as well,[16] but these

modalities are not widely available, particularly in resource constrained settings. Furthermore, measurements may be impossible in patients with severe obesity or ascites.[31] Reproducibility of transient elastography may be impaired in patients with steatosis, increased body mass index or less severe liver fibrosis.[32] In contrast, WFA$^+$ M2BP quantification requires a small blood sample and WFA$^+$ M2BP can be accurately measured without interference from the previously mentioned factors. WFA$^+$ M2BP quantification is entirely automated using the HISCL 2000i system and results can be acquired within 17 min. Because of these advantages, WFA$^+$ M2BP is more useful to predict liver fibrosis and HCC development than other serum fibrosis markers or elastography.

Our study was limited by the small number of patients and case control pilot design. Patient characteristics between two groups were matched, but age, sex and fibrosis stage were biased nevertheless. A larger prospective study is needed to evaluate the utility of WFA$^+$ M2BP and time course changes in WFA$^+$ M2BP as predictive factors of HCC development.

In conclusion, WFA$^+$ M2BP and time course changes in WFA$^+$ M2BP were found to be independent predictive factors of HCC development, and patients at high risk of HCC development could be identified by combining these factors into a scoring system. Because WFA$^+$ M2BP quantification can be easily repeated, real time monitoring of WFA$^+$ M2BP could be a novel predictor of HCC development.

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CONFLICT OF INTEREST

THE AUTHORS WHO have taken part in this study declare that they have no conflicts of interest to disclose.

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ORIGINAL ARTICLE

Reactivation of hepatitis B virus (HBV) infection in adult T-cell leukemia–lymphoma patients with resolved HBV infection following systemic chemotherapy

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Abstract Reactivation of hepatitis B virus (HBV) infection may occur in adult T-cell leukemia–lymphoma (ATL) patients with resolved HBV infection who receive monotherapy with the anti-CC chemokine receptor 4 monoclonal antibody, mogamulizumab. However, there is little evidence regarding the incidence and characteristics of HBV reactivation in ATL patients receiving systemic chemotherapy, including the use of this antibody. We conducted a retrospective study for 24 ATL patients with resolved HBV infection underwent regular HBV DNA monitoring to assess HBV reactivation in Nagoya City University Hospital between January 2005 and June 2013. With median HBV DNA follow-up of 238 days (range 57–1420), HBV reactivation (defined as the detection of HBV DNA) was observed in three (12.5 %) of 24 patients with resolved HBV infection. No hepatitis due to HBV reactivation occurred in those patients who were diagnosed with HBV DNA levels below 2.1 log copies/mL and who received antiviral drugs. Mogamulizumab was administered prior to HBV reactivation in two of three HBV-reactivated patients. In the mogamulizumab era, further well-designed prospective studies are warranted to estimate the incidence of HBV reactivation and to establish regular HBV DNA monitoring-guided preemptive antiviral therapy for such patients.

Keywords Reactivation · HBV · CCR4 · Mogamulizumab · ATL

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Abbreviations

HBV	Hepatitis B virus
ATL	Adult T-cell leukemia–lymphoma
HBsAg	Hepatitis B surface antigen
Anti-HBc	Antibodies against hepatitis B core antigen
Anti-HBs	Antibodies against hepatitis B surface antigen
CCR4	CC chemokine receptor 4

Introduction

Reactivation of hepatitis B virus (HBV) infection has been reported as a potentially fatal complication of systemic chemotherapy [1–6]. HBV reactivation may occur not only in hepatitis B surface antigen (HBsAg)-positive patients, but also in patients with resolved HBV infection who are seronegative for HBsAg but seropositive for antibodies

against hepatitis B core antigen (anti-HBc) and/or antibodies against HBsAg (anti-HBs).

Chemotherapy containing the anti-CD20 monoclonal antibody, rituximab plus steroids has been shown to be an important risk factor for HBV reactivation in B-cell lymphoma patients with resolved HBV infection [2, 3]. Recently, the anti-CC chemokine receptor 4 (CCR4) monoclonal antibody, mogamulizumab, was developed and introduced into the management of adult T-cell leukemia–lymphoma (ATL) [7–12]. A dose-finding study showed that mogamulizumab monotherapy could induce HBV reactivation-related hepatitis in an ATL patient with resolved HBV infection [9, 13].

However, there is little evidence regarding the incidence and characteristics of HBV reactivation in ATL patients with resolved HBV infection who were receiving systemic chemotherapy including this antibody. We conducted here a retrospective study in a single institution to evaluate the risk of HBV reactivation in these patients who underwent regular monitoring of HBV DNA levels during and after chemotherapy.

Patients and methods

Between January 2005 and June 2013, 66 patients were diagnosed with ATL in Nagoya City University Hospital. Baseline serological markers for HBsAg, anti-HBc, and anti-HBs were measured to evaluate their viral status before systemic chemotherapy. Antiviral prophylaxis was provided to the HBsAg-positive patients before the initiation of systemic chemotherapy. HBV DNA levels were assessed in HBsAg-negative patients who were seropositive for anti-HBc and/or anti-HBs. Patients seronegative for HBsAg but with detectable of HBV DNA were considered to have occult HBV infection, and antiviral prophylaxis was provided to those patients. HBsAg-negative patients seropositive for anti-HBc and/or anti-HBs but without detectable of HBV DNA were considered to have resolved HBV infection and their HBV DNA levels were monitored regularly (monthly in principle) for HBV DNA levels during chemotherapy and at least 1 year after chemotherapy; HBV reactivation was defined as the detection of HBV DNA. If HBV reactivation was confirmed, antiviral drugs were given immediately (preemptive antiviral therapy).

All baseline serological markers of HBsAg, anti-HBc and anti-HBs were measured by the laboratory in this hospital, using the following methods and cut-off values: CLEIA with cut-off values for HBsAg, anti-HBc and anti-HBs were 1.0 C.O.I, 1.0 INH % and 10.0 mIU/mL, respectively, from January 2005 to December 2010, CLEIA with cut-off values for HBsAg, anti-HBc and ant-HBs were 0.03 mIU/mL, 1.0 C.O.I, and 10.0 mIU/mL, respectively, from January 2011.

HBV DNA levels were measured by an outside laboratory (SRL, Inc.; Tokyo, Japan) or by the laboratory in this hospital, using the following methods and cut-off values: transcription-mediated amplification test with a cut-off value of 3.7 LGE/mL from January 2005 to April 2006, Amplicor HBV monitor test with a cut-off value of 2.6 log copies/mL from April 2006 to May 2008, COBAS AmpliPrep/COBAS TaqMan HBV test (v1.0) with a cut-off value of 1.8 log copies/mL from May 2008 to July 2009, and COBAS AmpliPrep/COBAS TaqMan HBV test (v2.0) with a cut-off value of 2.1 log copies/mL from July 2009.

For the analysis of HBV sequences, nucleic acids were extracted from the preserved serum specimens (200 μL) and subjected to PCR to amplify HBV genomes within the short S region [nucleotides (nt) 427–607] and the basal core promoter (BCP)/precore (PC) regions [nt 1628–2047] followed by direct sequencing using the ABI Prism Big Dye ver. 3.1 kit in an ABI 3100 DNA automated sequencer (Applied Biosystems, Foster City, CA). HBV genotypes were determined by molecular evolutionary analysis [14].

To compare the baseline characteristics and ATL treatment of the patients with and without HBV reactivation, we used the Chi-square test and two-sided Fisher's exact test for categorical data, and the Mann–Whitney U test for continuous variables. A two-tailed p value of less than 0.05 was considered statistically significant. All statistical analyses were performed using SPSS (version 22.0) statistical software for Windows, using data fixed on August 31, 2013. This study was approved by the Institutional Review Board of Nagoya City University. All patients gave written informed consent.

Results

The status of HBV infection at baseline was as follows (Fig. 1): HBsAg-positive (n = 2, 3.0 %), HBsAg-negative (n = 63, 95.5 %), and no serological HBV assessment (n = 1, 1.5 %). Of the 63 HBsAg-negative patients, 31 (49.2 %) were anti-HBc positive and/or anti-HBs positive. Of the remaining 32 patients, 31 were anti-HBc negative and anti-HBs negative, and one had no data for anti-HBc and anti-HBs. Because HBV DNA below 1.8 log copies/mL was detected at baseline in one patient who was anti-HBc positive and anti-HBs positive at baseline (and who was therefore judged to have occult HBV infection), antiviral drugs were administered before initiating systemic chemotherapy. Finally, 24 of 31 patients with resolved HBV infection underwent regular HBV DNA monitoring (Fig. 1). For these 24 ATL patients, initial systemic chemotherapy included the following regimens: CHOP (n = 7, 29.2 %), VCAP-AMP-VECP (n = 13, 54.2 %) and others (n = 4, 16.6 %) (Table 1). Systemic chemotherapy was started in 6

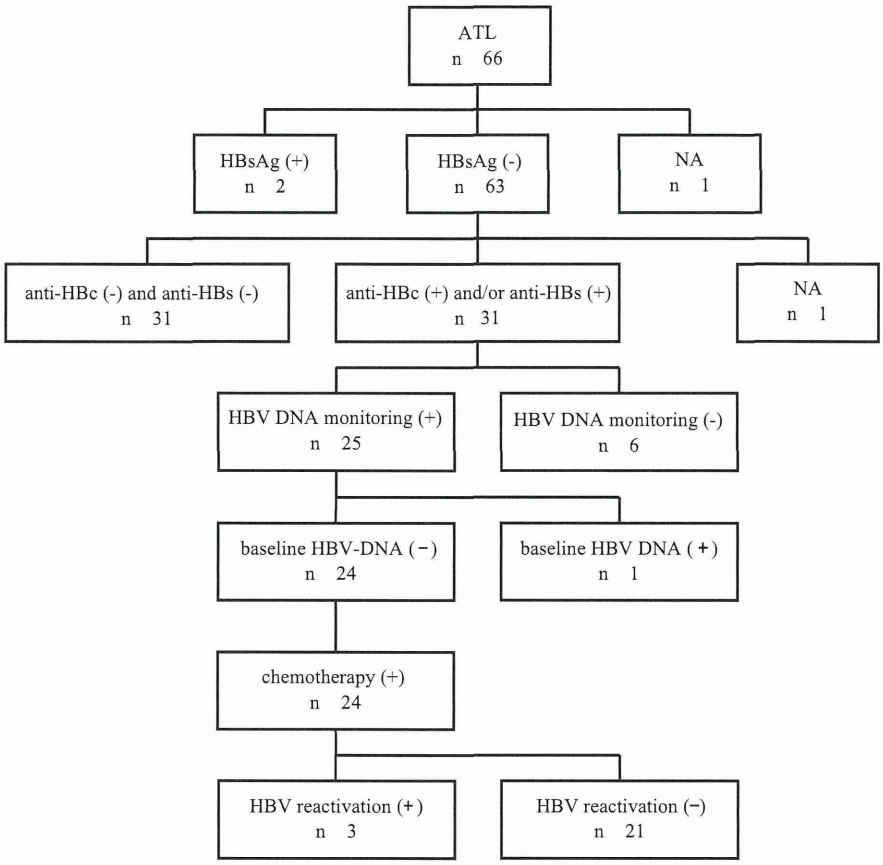

Fig. 1 Baseline serological markers of HBV infection in the 66 ATL patients. Two patients were HBsAg-positive, 63 were HBsAg-negative, the last was not available for serological HBV assessment. Of the 63 HBsAg-negative patients, 31 were anti-HBc-positive and/or anti-HBs-positive. One patient had detectable HBV DNA at baseline, and was judged as having occult HBV infection. Regular HBV DNA monitoring was performed in 24 of 31 patients with resolved HBV infection and 3 patients suffered HBV reactivation. *HBV* hepatitis B virus, *ATL* adult T-cell leukemia–lymphoma, *HBsAg* hepatitis B surface antigen, *anti-HBc* antibodies against hepatitis B core antigen, *anti-HBs* antibodies against hepatitis B surface antigen, *NA* not available

patients before HBV DNA monitoring. For the 24 patients with resolved HBV infection during and after systemic chemotherapy, regular monitoring of HBV DNA was conducted with a median interval of 30 days (range 2–703).

HBV reactivation was observed in 3 (12.5 %) of 24 patients with resolved HBV infection, with a median HBV DNA follow-up of 238 days (range 57–1420). No hepatitis due to HBV reactivation occurred in those patients who were diagnosed with HBV DNA levels below 2.1 log copies/mL and who received antiviral drugs (entecavir, 0.5 mg/day), resulting in no detectable HBV DNA levels during antiviral treatment.

There was no statistically significant difference in baseline characteristics and ATL treatment between patients with and without reactivation in this retrospective analysis (Table 1). The characteristics of 3 patients with HBV reactivation are shown in Table 2; all were male, and seropositive for anti-HBc and anti-HBs at baseline, and received the VCAP-AMP-VECP regimen as initial treatment. Mogamulizumab was administered prior to HBV reactivation in 2 of 3 HBV-reactivated patients. The anti-HBs titers of 3 patients decreased at reactivation compared to baseline titers in 3 patients. Their HBV genotypes were determined as C. HBV mutations were not found in the precore region or basal core promoter. One patient died due to ATL progression.

The clinical course of case 1 is shown in Fig. 2. HBV reactivation was confirmed with HBV DNA levels below 2.1 log copies/mL, 3 months after initiating mogamulizumab-containing chemotherapy as initial treatment for ATL. The patient presented with elevation of transaminase levels after detection of HBV DNA, it considered not viral hepatitis, but drug-induced liver damage because of transient and slight increase of HBV DNA levels. Reemergence of HBV was observed repeatedly after withdrawal of antiviral drugs following the development of drug-induced allergic rash or interstitial pneumonia. The patient maintains complete remission of ATL with undetectable of HBV DNA after withdrawal of antiviral drugs over 3 years after mogamulizumab-containing chemotherapy.

Discussion

This study showed that the incidence of HBV reactivation among ATL patients with resolved HBV infection who received systemic chemotherapy was 12.5 %. Preemptive antiviral therapy, guided by regular HBV DNA monitoring,