25 Yamaura T, Tanaka E, Matsumoto A *et al.* A case-control study for early prediction of hepatitis B e antigen seroconversion by hepatitis B virus DNA levels and mutations in the precore region and core promoter. *J Med Virol* 2003; **70:** 545–52.
26 Brunetto MR, Oliveri F, Colombatto P *et al.* Hepatitis B surface antigen serum levels help to distinguish active from inactive hepatitis B virus genotype D carriers. *Gastroenterology* 2010; **139:** 483–90.
27 Nakazawa T, Shibuya A, Takeuchi A *et al.* Viral level is an indicator of long-term outcome of hepatitis B virus e antigen-negative carriers with persistently normal serum alanine aminotransferase levels. *J Viral Hepat* 2011; **18:** e191–9.
28 Togo S, Arai M, Tawada A *et al.* Clinical importance of serum hepatitis B surface antigen levels in chronic hepatitis B. *J Viral Hepat* 2011; **18:** e508–15.
29 Park H, Lee JM, Seo JH *et al.* Predictive value of HBsAg quantification for determining the clinical course of genotype C HBeAg-negative carriers. *Liver Int* 2012; **32:** 796–802.
30 Chen YC, Huang SF, Chu CM, Liaw YF. Serial HBV DNA levels in patients with persistently normal transaminase over 10 years following spontaneous HBeAg seroconversion. *J Viral Hepat* 2012; **19:** 138–46.
31 Kimura T, Ohno N, Terada N *et al.* Hepatitis B virus DNA-negative dane particles lack core protein but contain a 22-kDa precore protein without C-terminal arginine-rich domain. *J Biol Chem* 2005; **280:** 21713–19.
32 Shinkai N, Tanaka Y, Orito E *et al.* Measurement of hepatitis B virus core-related antigen as predicting factor for relapse after cessation of lamivudine therapy for chronic hepatitis B virus infection. *Hepatol Res* 2006; **36:** 272–6.
33 Matsumoto A, Tanaka E, Minami M *et al.* Low serum level of hepatitis B core-related antigen indicates unlikely reactivation of hepatitis after cessation of lamivudine therapy. *Hepatol Res* 2007; **37:** 661–6.
34 Brunetto MR, Moriconi F, Bonino F *et al.* Hepatitis B virus surface antigen levels: a guide to sustained response to peginterferon alfa-2a in HBeAg-negative chronic hepatitis B. *Hepatology* 2009; **49:** 1141–50.
35 Moucari R, Mackiewicz V, Lada O *et al.* Early serum HBsAg drop: a strong predictor of sustained virological response to pegylated interferon alfa-2a in HBeAg-negative patients. *Hepatology* 2009; **49:** 1151–7.
36 McMahon BJ. The influence of hepatitis B virus genotype and subgenotype on the natural history of chronic hepatitis B. *Hepatol Int* 2009; **3:** 334–42.
37 Papatheodoridis GV, Manolakopoulos S, Liaw YF, Lok A. Follow-up and indications for liver biopsy in HBeAg-negative chronic hepatitis B virus infection with persistently normal ALT: a systematic review. *J Hepatol* 2012; **57:** 196–202.

Hepatology Research

Hepatology Research 2013; 43: 596–604 doi: 10.1111/j.1872-034X.2012.01115.x

Original Article

Fibrosis score consisting of four serum markers successfully predicts pathological fibrotic stages of chronic hepatitis B

Kenji Ikeda,[1,2] Namiki Izumi,[3] Eiji Tanaka,[7] Hiroshi Yotsuyanagi,[4] Yoshihisa Takahashi,[5] Junichi Fukushima,[6] Fukuo Kondo,[5] Toshio Fukusato,[5] Kazuhiko Koike,[4] Norio Hayashi[8] and Hiromitsu Kumada[1,2]

[1]*Department of Hepatology, Toranomon Hospital,* [2]*Okinaka Memorial Institute for Medical Research,* [3]*Department of Gastroenterology, Musashino Red Cross Hospital,* [4]*Department of Gastroenterology, Tokyo University of Medicine,*[5]*Department of Pathology, Teikyo University School of Medicine,* [6]*Department of Pathology, NTT Medical Center Tokyo, Tokyo,* [7]*Department of Gastroenterology, Shinshu University of Medicine, Matsumoto, and* [8]*Department of Gastroenterology, Kansai-Rosai Hospital, Hyogo, Japan*

Aim: In order to evaluate and judge a fibrotic stage of patients with chronic hepatitis B, multivariate regression analysis was performed using multiple fibrosis markers.

Method: A total of 227 patients from seven hepatology units and institutes were diagnosed by needle biopsy as having chronic liver disease caused by hepatitis B virus. Twenty-three variables and their natural logarithmic transformation were employed in the multivariate analysis. Multiple regression function was generated from data of 158 patients in one hospital, and validation was performed using the other data of 69 patients from six other hospitals.

Results: After stepwise variable selection, multivariate regression analysis finally obtained the following function: $z = 1.40 \times ln$ (type IV collagen 7S) (ng/mL) $- 0.017 \times$ (platelet count) ($\times 1000^3/mm^3$) $+ 1.24 \times ln$ (tissue inhibitor of matrix metalloproteinase-2) (ng/mL) $+ 1.19 \times ln$ (α-2-macroglobulin) (mg/dL) $- 9.15$. Median values of fibrosis scores of F1 ($n = 73$), F2 ($n = 42$), F3 ($n = 31$) and F4 stages ($n = 12$) were calculated as 0.95, 2.07, 2.98 and 3.63, respectively. Multiple regression coefficient and coefficient of determination were 0.646 and 0.418, respectively. Validation with patient data from other institutions demonstrated good reproducibility of fibrosis score for hepatitis B (FSB), showing 1.33 in F1 ($n = 27$), 2.20 in F2 ($n = 20$), 3.11 in F3 ($n = 20$) and 5.30 in F4 ($n = 2$), respectively.

Conclusion: A concise multiple regression function using four laboratory parameters successfully predicted pathological fibrosis stage of patients with hepatitis B virus infection.

Key words: chronic hepatitis, hepatitis B virus, liver cirrhosis, liver fibrosis, multiple regression analysis, stage

INTRODUCTION

WHEN HEPATITIS B virus (HBV)-related chronic liver disease is found by biochemical and virological examination, liver biopsy can establish the definitive diagnosis of chronic hepatitis and its fibrotic staging. Although these pathological procedures are reliable and informative both in diagnosis and treatment, they sometimes require medical invasion and financial costs, including the risk of bleeding from needle puncture, some pain experienced during the procedure and hospital stays of a few days. The pathological examination is, therefore, rarely performed repeatedly in a short period of time, unless disease activity is severe or progression of liver disease is highly suspected. Recently, many authors described the usefulness of ultrasonographic elastography and multiple resonance imaging technology in the estimation of staging of chronic hepatitis and cirrhosis.[1–5] These ways of estimation using the imaging apparatuses seem truly useful for current patients, but they cannot evaluate and compare with past fibrotic states of patients retrospectively. Moreover,

Correspondence: Dr Kenji Ikeda, Department of Hepatology, Toranomon Hospital, 2-2-2 Toranomon, Minato-ku, Tokyo, 105-8470, Japan. Email: ikedakenji@tora.email.ne.jp
Received 6 May 2012; revision 17 September 2012; accepted 4 October 2012.

the same apparatus for elastometry will not be available for repeated measurement for a follow-up examination, for example, several years later.

In spite of the accuracy of biopsy and convenience of elastography in chronic liver disease, clinical diagnosis based on biochemistry and hematology is still indispensable for the daily practice of many patients with HBV-related liver disease. Recently, several studies were published about estimation of hepatitis stages, using one or more serum biomarkers. Discriminant functions or multivariate analyses demonstrated that approximately 60–90% of patients with chronic hepatitis B were correctly classified as having mild hepatitis and severe hepatitis with advanced fibrosis.[2,6–13] Up to the present time, however, the usefulness of the discriminant functions are less valuable for a few reasons. First, these functions were made for the purpose of discrimination of severe hepatic fibrosis from mild fibrosis, and four histological classifications (F1–F4) were neglected in almost of the studies. Second, some studies analyzed both hepatitis B and hepatitis C virus infection, although the significance and actual values of each liver function test in the evaluation of the severity of liver disease were not similar among each viral hepatitis and alcoholic liver disease. Third, biochemical markers for liver fibrosis (e.g. hyaluronic acid, type IV collagen, procollagen III peptide)[14–16] were not always included in those previous studies.

We tried to generate a function estimating fibrotic stages of HBV-related chronic hepatitis, which were objectively diagnosed by liver biopsy. The purpose of this study is, therefore, to make a reliable multiple regression function and to obtain practical coefficients for significant variables also using fibrosis markers.

METHODS

Patients

A TOTAL OF 273 Japanese patients with chronic hepatitis B were recruited for the study from seven hospitals in Japan: Toranomon Hospital, Hiroshima University Hospital (K. Chayama, M.D.), Ehime University Hospital (M. Onji, M.D.), Musashino Red Cross Hospital (N. Izumi, MD), Shishu University Hospital (E. Tanaka, M.D.), Showa University Hospital (M. Imawari, M.D.) and Osaka University Hospital (T. Takehara, M.D.). Inclusion criteria for this study were: (i) positive hepatitis B surface antigen for more than 6 months; (ii) persistent or intermittent elevation in aspartate aminotransferase (AST)/alanine aminotransferase (ALT) levels; and (iii) liver biopsy showing chronic hepatitis (F1–F4). We excluded those patients with overt alcoholic liver disease or fatty liver, association of other types of liver disease (e.g. hepatitis C, primary biliary cirrhosis, autoimmune hepatitis), or those associated with hepatocellular carcinoma or other malignancy. Among the patients, 244 patients fulfilled the conditions for the study: complete demographic data, basic laboratory data of hematology and biochemistry, required liver biopsy specimens, and sufficient amount of frozen sera. Also, we excluded additional 17 patients with eventual histological diagnosis as F0 stage.

Finally, a total of 227 patients who were diagnosed as having chronic hepatitis or cirrhosis (F1–F4) were analyzed for the following hematological, biochemical and histopathological examination. There were 172 males and 55 females aged 16–70 years (median, 39 years).

All the patients presented written informed consent in individual hospitals and medical centers, and the study was approved in each ethical committee.

Hematological and biochemical examination

Hematological and standard biochemical evaluation had been performed in each medical institution: white blood cells, red blood cells, hemoglobin, platelets, total bilirubin, AST, ALT, AST/ALT ratio (AAR), γ-glutamyl transpeptidase (γ-GTP), total protein, albumin and γ-globulin.

Special biochemical examinations including "fibrosis markers" were carried out using stored frozen sera at −20°C or lower: α-2-macroglobulin, haptoglobin concentration, haptoglobin typing, apolipoprotein A1, hyaluronic acid, tissue inhibitor of matrix metalloproteinase (TIMP)-1, TIMP-2, procollagen III peptide and type IV collagen 7S.

Histological diagnosis of chronic hepatitis and cirrhosis

All the 227 cases fulfilled required standards of histological evaluation: sufficient length of specimen, hematoxylin–eosin staining, and at least one specimen with fiber staining. Four independent pathologists (Y. T., J. F., F. K. and T. F.), who were not informed of patients' background and laboratory features except for age and sex, evaluated the 227 specimens regarding the stages of fibrosis and activity. Pathological classification of chronic hepatitis staging was based on Desmet *et al.*[17]

Before judgment of histological staging of individual specimens, the pathologists discussed the objective and reproducible judgment of pathological diagnosis of

hepatitis. They made a panel about obvious criteria using typical microscopic pictures for each stage, and it was always referred to during the procedure of pathological judgment. When inconsistent results were found in the diagnosis of hepatitis stage among the pathologists, the final judgment accepted majority rule among them.

Statistical analysis

Non-parametric procedures were employed for the analysis of background characteristics and laboratory data among patients in each stage, including Mann–Whitney *U*-test, Kruskal–Wallis test and χ^2-test.

The normality of the distribution of the data was evaluated by a Kolmogorov–Smirnov one-sample test. Because certain variables partly did not conform to a normal distribution, natural logarithmic transformation of bilirubin, AST, ALT, γ-GTP, α-2-macroglobulin, hyaluronic acid, type IV collagen 7S and TIMP-2 were also analyzed in the following calculation. The natural logarithmic transformation of the results yielded a normal distribution or symmetrical distribution for all the analyzed factors. After the procedures, the following multiple regression analysis became rationally robust against deviations from normal distribution. In order to avoid introducing into the model any variables that were mutually correlated, we checked the interaction between all pairs of the variables by calculating variance inflation factors. Of the highly correlated variables, less significant factors were removed from the viewpoint of multicollinearity.

Multivariate regression analysis was performed using 158 patient data from Toranomon Hospital (training dataset) to generate a training data of predicting function. We used a stepwise method for selection of informative subsets of explanatory variables in the model. Multiple regression coefficient and coefficient of determination were also taken into account in the selection of variables. Next, we validated the obtained predictive function using the remaining 69 patient data from the other six liver institutions (validation dataset).

A *P*-value of less than 0.05 with two-tailed test was considered to be significant. Data analysis was performed using the computer program SPSS ver. 19.[18]

For evaluation of the efficiency and usefulness of obtained function for fibrosis estimation, we compared various fibrosis scores for hepatitis B and C, including AAR,[19] AST-to-platelet ratio index (APRI),[20] FIB-4,[21] FibroTest[22] and discrimination function of cirrhosis from hepatitis in Japanese patients.[23]

RESULTS

Pathological diagnosis

FOUR PATHOLOGISTS INDEPENDENTLY judged the fibrotic stages and inflammatory activity for 227 specimens of chronic hepatitis/cirrhosis caused by HBV. One hundred patients (44.1%) had a fibrosis stage of F1, 62 (27.3%) F2, 51 (22.5%) F3 and 14 (6.2%) F4. In the subgroup of the 158 patients in the training group, judgment as F1 was made in 73 cases, F2 in 42, F3 in 31 and F4 in 12. Of the 69 patients in the validation group, judgment as F1 was made in 27, F2 in 20, F3 in 20 and F4 in two.

According to hepatitis activity classification, A0 was found in five (2.2%), A1 in 100 (44.1%), A2 in 107 (47.1%) and A3 in 15 (6.6%).

Laboratory data of each hepatitis stage in the training group

There were 124 men and 34 women with a median age of 39 years ranged 16–70 years. Laboratory data of 158 patients in the training group are shown in Table 1. Although several individual items were well correlated with the severity of hepatic fibrosis, significant overlap values were noted among F1–F4 stages: platelet count, γ-globulin, α-2-macroglobulin, haptoglobin, hyaluronic acid, TIMP-2 and type IV collagen 7S.

Significant variables serving staging of hepatitis

Univariate analyses using trend analysis with the Cochran–Armitage method showed that the fibrotic stage of chronic hepatitis B (FSB) was significantly correlated with platelet count (Spearman: $r = -0.45$, $P < 0.001$), γ-GTP ($r = 0.19$, $P = 0.017$), γ-globulin ($r = 0.29$, $P < 0.001$), α-2-macroglobulin ($r = 0.32$, $P < 0.001$), hyaluronic acid ($r = 0.36$, $P < 0.001$), TIMP-2 ($r = 0.16$, $P = 0.043$), procollagen III peptide ($r = 0.30$, $P < 0.001$) and type IV collagen 7S ($r = 0.55$, $P < 0.001$).

Regression function generated from training patient group

After stepwise variable selection, multivariate regression analysis finally obtained the following function: $z = 1.40 \times ln$ (type IV collagen 7S) (ng/mL) $- 0.017 \times$ (platelet count) ($\times 1000^3/mm^3$) $+ 1.24 \times ln$ (TIMP-2) (ng/mL) $+ 1.19 \times ln$ (α-2-macroglobulin) (mg/dL) $-$ 9.15. Median values of the fibrosis score of F1 ($n = 73$), F2 ($n = 42$), F3 ($n = 31$) and F4 stages ($n = 12$) were calculated as 0.95, 2.07, 2.98 and 3.63, respectively

Table 1 Demography and laboratory data of 158 patients in training group

	F1 ($n = 73$)	F2 ($n = 42$)	F3 ($n = 31$)	F4 ($n = 12$)
Demographics				
Men : women	58:15	33:9	23:8	10:2
Age (median, range)	36 (16–70)	39.5 (18–66)	39 (25–64)	43 (32–59)
Laboratory data (median, range)				
WBC (×1000/mm^3)	5.4 (2.5–10.6)	5.1 (2.4–8.7)	4.9 (3.0–8.7)	4.1 (3.7–6.6)
Hemoglobin (g/dL)	15.3 (10.3–18.8)	15.4 (12.5–17.9)	15.2 (11.5–17.2)	14.45 (12.1–18.2)
Platelet (×1000/mm^3)	204 (124–341)	173 (82–308)	155 (96–220)	130 (86–230)
Albumin (g/dL)	4.1 (3.2–4.9)	4.0 (3.2–5.1)	4.0 (3.3–4.9)	3.95 (3.4–4.6)
Bilirubin (mg/dL)	0.8 (0.2–1.7)	0.8 (0.3–2.3)	0.9 (0.4–5.4)	0.85 (0.6–2.3)
AST (IU/L)	48 (16–450)	55 (17–588)	54 (17–1446)	76.5 (27–396)
ALT (IU/L)	102 (10–839)	90 (12–886)	85 (19–2148)	89 (18–809)
γ-GTP (IU/L)	37 (7–247)	55 (8–687)	44 (14–564)	69 (33–262)
γ-Globulin (g/dL)	1.29 (0.78–2.11)	1.495 (0.62–3.20)	1.43 (0.90–2.30)	1.735 (0.92–2.47)
γ-Globulin (%)	17.3 (10.8–26.1)	19.3 (8.5–35.6)	19.9 (12.9–28.6)	22.55 (13.9–30.2)
α-2-Macroglobulin (mg/dL)	226 (116–446)	276 (148–495)	261 (202–565)	286.5 (166–425)
Haptoglobin (mg/dL)	77 (<5–318)	59 (<5–238)	61 (<5–151)	48.5 (<5–145)
Apolipoprotein A-I (mg/dL)	134 (89–212)	143 (78–250)	133 (87–189)	125 (73–169)
Hyaluronic acid (μg/L)	16 (<5–130)	32.5 (<5–204)	38 (<5–418)	49 (24–335)
TIMP-1 (ng/mL)	168 (93–271)	172 (116–314)	157 (119–365)	192 (145–365)
TIMP-2 (ng/mL)	80 (41–135)	80.5 (35–121)	92 (38–251)	85.5 (70–123)
Procollagen III peptide (U/mL)	0.75 (0.53–1.90)	0.835 (0.45–1.20)	0.89 (0.58–2.50)	1.05 (0.71–2.20)
Type IV collagen 7S (ng/ml)	4.0 (2.7–7.7)	4.6 (2.6–9.6)	5.6 (2.3–15.0)	7.2 (4.2–14.0)

ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GTP, γ-glutamyl transpeptidase; TIMP, tissue inhibitor of matrix metalloproteinase; WBC, white blood cells.

(Fig. 1). The multiple regression coefficient and coefficient of determination were 0.646 ($P < 0.001$) and 0.418 ($P < 0.001$), respectively.

Because the generated regression function was obtained by multivariate analysis with stepwise variable selection, several variables were removed from the function due to multicollinearity among them. Mutual correlation among the fibrosis predictors are shown in Table 2.

A 28-year-old man of F1 fibrotic stage (Fig. 2a) had a serum type IV collagen concentration of 4.4 ng/mL, platelet 221 × 10^3 count/mm^3, TIMP-2 75 ng/mL and α-2-macroglobulin 226 mg/dL. The regression function provided a fibrosis score of 0.99. Another man aged 46 years had F3 fibrosis on histological examination (Fig. 2b). His type IV collagen was 5.3 ng/mL, platelet 137 × 10^3 count/mm^3, TIMP-2 92 ng/mL and α-2-macroglobulin 255, and the regression function calculated his fibrosis score as 3.10.

Validation of discriminant function

Validation data of 69 patients (Table 3) were collected from the other six institutions in Japan. When applying

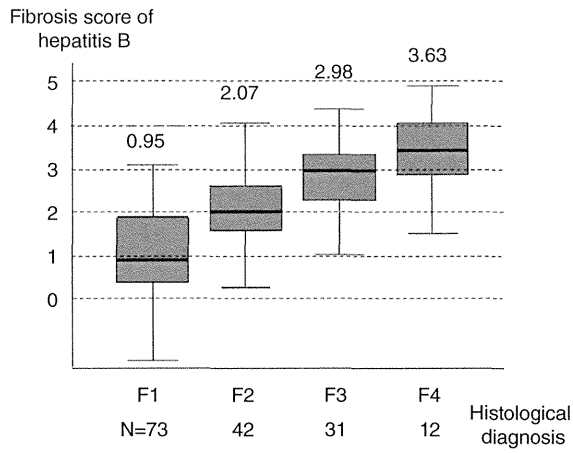

Figure 1 Box and whisker plots of fibrotic score of each histological fibrosis group in the training dataset. The fibrosis score of hepatitis B was generated by the function, z = 1.40 × *ln* (type IV collagen 7S) (ng/mL) − 0.017 × (platelet count) (×1000^3/mm^3) + 1.24 × *ln* (tissue inhibitor of matrix metalloproteinase-2) (ng/mL) + 1.19 × *ln* (α-2-macroglobulin) (mg/dL) − 9.15.

Table 2 Correlation coefficients (Spearman's ρ) among fibrosis predictors used in multivariate analysis

	Platelet	gamma-globulin	*ln* (α-2-macroglobulin)	*ln* (hyaluronate)	*ln* (P-III-P)	*ln* (IV collagen)	*ln* (TIMP-2)
Platelet ($\times 10^3/mm^3$)	1.000	−0.214 ($P = 0.008$)	−0.260 ($P = 0.001$)	−0.384 ($P < 0.001$)	−0.045 ($P = 0.58$)	−0.297 ($P < 0.001$)	0.094 ($P = 0.24$)
γ-Globulin (g/dL)		1.000	0.276 ($P = 0.001$)	0.349 ($P < 0.001$)	0.342 ($P < 0.001$)	0.414 ($P < 0.001$)	0.268 ($P = 0.001$)
ln (α-2-macroglobulin) (mg/dL)			1.000	0.281 ($P < 0.001$)	0.141 ($P = 0.078$)	0.171 ($P = 0.032$)	−0.079 ($P = 0.32$)
ln (hyaluronic acid) (mg/L)				1.000	0.373 ($P < 0.001$)	0.493 ($P < 0.001$)	0.089 ($P = 0.27$)
ln (procollagen III peptide) (U/mL)					1.000	0.600 ($P < 0.001$)	0.145 ($P = 0.071$)
ln (type IV collagen) (mg/L)						1.000	0.358 ($P < 0.001$)
ln (TIMP-2) (mg/L)							1.000

TIMP, tissue inhibitor of matrix metalloproteinase.

the regression function for the validation set, the fibrosis score demonstrated good reproducibility, showing 1.33 in patients with chronic hepatitis of F1 ($n = 27$), 2.20 of F2 ($n = 20$), 3.11 of F3 ($n = 20$) and 5.30 of F4 ($n = 2$), respectively (Fig. 3). Although F4 fibrosis stage consisted of only two patients and the score 5.30 was regarded as of rather higher value, the scores of other stages of fibrosis were concordant with histological fibrosis.

Comparisons of efficacy with various fibrosis scores (Fig. 4)

In order to evaluate the efficacy and usefulness of the obtained FSB, we compared it with previously reported fibrosis scores using training data. AAR, APRI and FibroTest showed only slight correlation with actual histological stage. FIB-4 demonstrated an increasing trend of the score associated with histological fibrosis, but significant overlapping scores were found in F1–F4. Spearman's correlation coefficients of AAR, APRI, FIB-4 and FibroTest were 0.199 ($P = 0.012$), 0.265 ($P = 0.001$), 0.412 ($P < 0.001$) and 0.330 ($P < 0.001$), respectively. Our FSB showed a Spearman's correlation coefficient of 0.625 ($P < 0.001$), and was a much higher value than the others. The dichotomous discrimination function for cirrhosis and hepatitis C in Japanese patients[23] showed good differentiation also in patients with hepatitis B virus.

DISCUSSION

RECOGNITION OF SEVERITY of chronic hepatitis is essential in managing patients with chronic HBV infection: estimation of length of infection, existence of any previous hepatitis activity, presumption of current fibrotic stage, and prediction of future fibrosis progression and hepatocarcinogenesis. Differential diagnosis of cirrhosis from chronic hepatitis is especially important in the evaluation of chronic HBV infection. Identification of liver cirrhosis often leads to an important change in management of the patient: need for fiberscopic examination for esophageal varices, ultrasonographic exploration for the association of liver cancer, and prediction of hepatic decompensation. Guidelines published by the American Association of Study of Liver Disease[24] recommend liver biopsy for HBV carriers with aminotransferase elevation or for any candidates of antiviral therapy, because hepatic fibrosis sometimes shows unexpectedly far advancement to cirrhosis, and because it is very difficult to evaluate and translate the liver function tests or ultrasonographic findings compared to chronic hepatitis type C.

Table 3 Demography and laboratory data of 69 patients in training group

	F1 ($n = 27$)	F2 ($n = 20$)	F3 ($n = 20$)	F4 ($n = 2$)
Demographics				
Men : women	18:9	15:5	13:7	2:0
Age (median, range)	36 (13–64)	45 (14–64)	36.5 (24–59)	32 (25–39)
Laboratory data (median, range)				
WBC (×1000/mm^3)	5.0 (2.8–8.7)	5.8 (2.8–11.6)	5.3 (3.2–8.1)	3.85 (2.7–5.0)
Hemoglobin (g/dL)	14.8 (12.4–17.4)	15.0 (12.4–16.9)	14.4 (11.1–16.4)	14.4 (12.5–16.3)
Platelet (×1000/mm^3)	204 (86–322)	180 (90–275)	147 (90–276)	130 (67–183)
Albumin (g/dL)	4.4 (2.8–5.2)	4.2 (3.5–5.1)	4.3 (3.4–4.9)	4.45 (4.0–4.9)
Bilirubin (mg/dL)	0.9 (0.4–6.4)	0.8 (0.2–1.6)	0.75 (0.4–1.7)	1.15 (1.1–1.2)
AST (IU/L)	52 (17–575)	50.5 (21–272)	65 (22–284)	248.5 (51–446)
ALT (IU/L)	84 (16–1101)	101.5 (19–554)	86.5 (16–1113)	453.5 (74–833)
γ-GTP (IU/L)	42 (14–332)	54 (16–205)	52.5 (13–191)	193 (57–329)
γ-Globulin (g/dL)	1.30 (1.04–1.59)	1.35 (1.18–2.53)	1.62 (1.16–1.97)	1.545 (1.51–1.58)
γ-Globulin (%)	17.9 (14.3–22.1)	19.6 (15.5–30.8)	22.0 (16.5–24.6)	20.15 (19.3–21.0)
α-2-Macroglobulin (mg/dL)	287 (160–687)	270 (89–452)	272.5 (211–463)	389 (313–465)
Haptoglobin (mg/dL)	58 (<5–229)	74 (<5–154)	56.5 (<5–198)	<5 (<5–<5)
Apolipoprotein A-I (mg/dL)	146 (95–216)	137 (87–162)	120 (88–170)	100.5 (74–127)
Hyaluronic acid (μg/L)	27 (<5–113)	36 (10–1050)	59 (14–439)	331 (225–437)
TIMP-1 (ng/mL)	168.5 (83–302)	176 (127–408)	182 (104–303)	390.5 (283–498)
TIMP-2 (ng/mL)	76 (25–143)	86.5 (28–154)	77.5 (32–141)	100.5 (91–110)
Procollagen III peptide (U/mL)	0.71 (0.27–2.20)	0.88 (0.63–2.80)	0.995 (0.60–2.10)	1.75 (1.50–2.00)
Type IV collagen 7S (ng/ml)	3.6 (2.7–17.0)	5.25 (3.3–13.0)	5.7 (3.0–16.0)	15.5 (15.0–16.0)

ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GTP, γ-glutamyl transpeptidase; TIMP, tissue inhibitor of matrix metalloproteinase; WBC, white blood cells.

Recently, non-invasive estimation of severity of liver fibrosis has been reported in patients with HBV-related chronic hepatitis.[2,6–13] However, these studies were principally aimed at differentiation of advanced fibrotic stages of F3 or F4 from mild fibrotic stages of F1 or F2. Those discrimination functions were insufficient to recognize the stepwise progression of viral hepatitis from F1–F4. This dichotomy (mild or severe) of chronic hepatitis B seemed less valuable in the study of disease progression, disease control abilities of antiviral drugs and estimation of histological improvement after anti-inflammatory drugs. A histology-oriented, practical and reliable formula is therefore required for the diagnosis and investigation of chronic hepatitis B.

This study aimed to establish non-invasive evaluation and calculation of liver fibrosis for patients with chronic hepatitis B virus infection. Although it was retrospectively performed as a multicenter study of eight institutions, judgment of histological diagnosis was independently performed by four pathologists in another hospital, who were informed only of the patient's age, sex and positive HBV infection. Objective judgment of the histological staging and grading in sufficient biopsy specimens could be obtained.

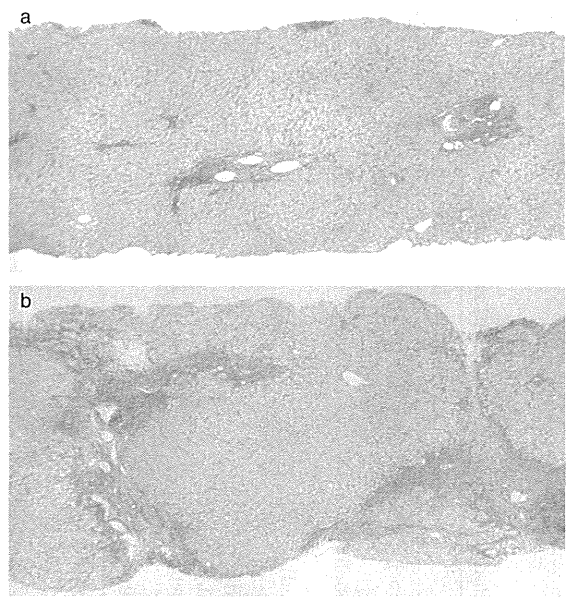

Figure 2 Case presentations of the training set. (a) A 28-year-old man with F1 fibrosis. Final regression function provided his fibrosis score as 0.99. (b) A 45-year-old man with F3 fibrosis. His regression coefficient was calculated as 3.10. Silver stain, ×40.

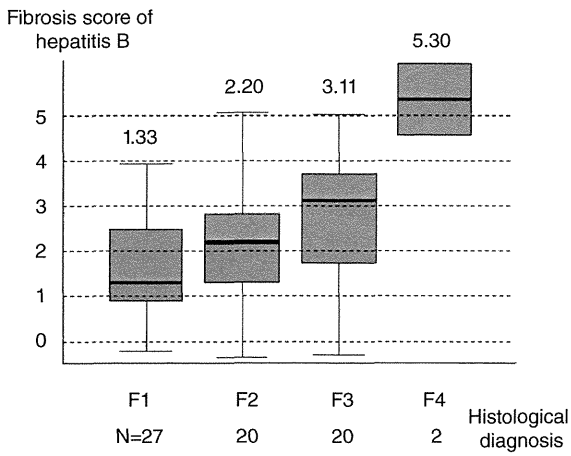

Figure 3 Box and whisker plots of fibrotic score of each group of histological fibrosis in the validation dataset. The fibrosis score of hepatitis B was generated by the function, $z = 1.40 \times ln$ (type IV collagen 7S) (ng/mL) $- 0.017 \times$ (platelet count) ($\times 1000^3/mm^3$) $+ 1.24 \times ln$ (tissue inhibitor of matrix metalloproteinase-2) (ng/mL) $+ 1.19 \times ln$ (α-2-macroglobulin) (mg/dL) $- 9.15$.

As many as 227 patients with chronic hepatitis B were analyzed in this study, who had been diagnosed as having chronic hepatitis or cirrhosis by liver biopsy performed in experienced liver units in Japan. To obtain the most suitable equation approximating histological fibrotic stage, multivariate analysis was performed using two demographic parameters (age and sex) and 21 hematological and biochemical markers with or without logarithmic transformation. They included many kinds of fibrosis markers: α-2-macroglobulin, haptoglobin concentration, haptoglobin typing, apolipoprotein A1, hyaluronic acid, TIMP-1, TIMP-2, procollagen III peptide and type IV collagen 7S Multiple regression analysis finally generated a first-degree polynomial function consisting of four variables: type IV collagen 7S, platelet count, TIMP-2 and α2-macroglobulin. A constant numeral (−9.15) was finally adjusted in the regression equation in order to obtain fitted figures for a fibrotic stage of F1–F4. From the magnitude of the standardized partial regression coefficient of individual variable in the function, platelet count demonstrated the most potent contribution toward the prediction of liver fibrosis. Type IV collagen 7S and *ln* (TIMP-2) proved to be the second and third distinctive power in the model, respectively.

The FSB was sufficiently fitted to actual fibrotic stages with certain overlapping as is usually found in histological ambiguity judged by pathologists. Because judgment of fibrosis in chronic hepatitis often shows a transitional histological staging, pathological examination cannot always make a clear-cut diagnosis discriminating F1–F4. Considering the limitation of the pathological difficulty in differentiating the four continuous disease entities, the obtained regression function showed satisfactory high accuracy rates in the prediction of liver disease severity. The FSB can provide one or two decimal places (e.g. 3.2 or 3.24) and the utility of the score is possibly higher than the mere histological stage of F1–F4. The reproducibility was confirmed by the remaining 67 patients' data obtained from the other six hospitals. Although the validation data were collected from a different geographic area and different chronological situation, the FSB showed similar results in prediction of histological staging.

The FSB seemed a very useful quantitative marker in evaluating fibrotic severity of hepatitis B patients without invasive procedures and without any specialized ultrasonography or magnetic resonance imaging. The FSB also has an advantage of measurement, in which old blood samples are available for retrospective assessment of varied clinical settings: for example, old sera from 20 years prior to the time of initial liver biopsy, or paired sera before and after long-term antiviral therapy. These kinds of retrospective assessments of fibrotic staging will be valuable in estimating a long-term progression of liver disease, in evaluating efficacy of long-term medication or other medical intervention, or in making a political judgment from the viewpoints of socioeconomic efficacy.

The score can be calculated for any patients with chronic HBV infection. Although this multiple regression model dealt with appropriate logarithmic transformation for non-normal distribution parameters, the regression analysis was based on a linear regression model. Very slight fibrosis can be calculated as less than 1.00, which is commonly found to a slight degree in chronic hepatitis with tiny fibrotic change as F0. Very severe fibrosis might be calculated as more than 4.00, which is an imaginary and nonsense number in the scoring system of fibrosis. The FSB is, however, very useful and valuable in a real clinical setting: estimation of severity of liver fibrosis in an outpatient clinic, evaluation of the natural progression of a patient's fibrosis over 10 years and assessment of a long-term administration of interferon in patients with chronic hepatitis B from the viewpoint of fibrotic change. Recent development of new nucleoside/nucleotide analogs requires evaluation for long-term histological advantage, for aggravation of hepatitis stage during viral and biochemical breakthrough caused by HBV mutation, and even for

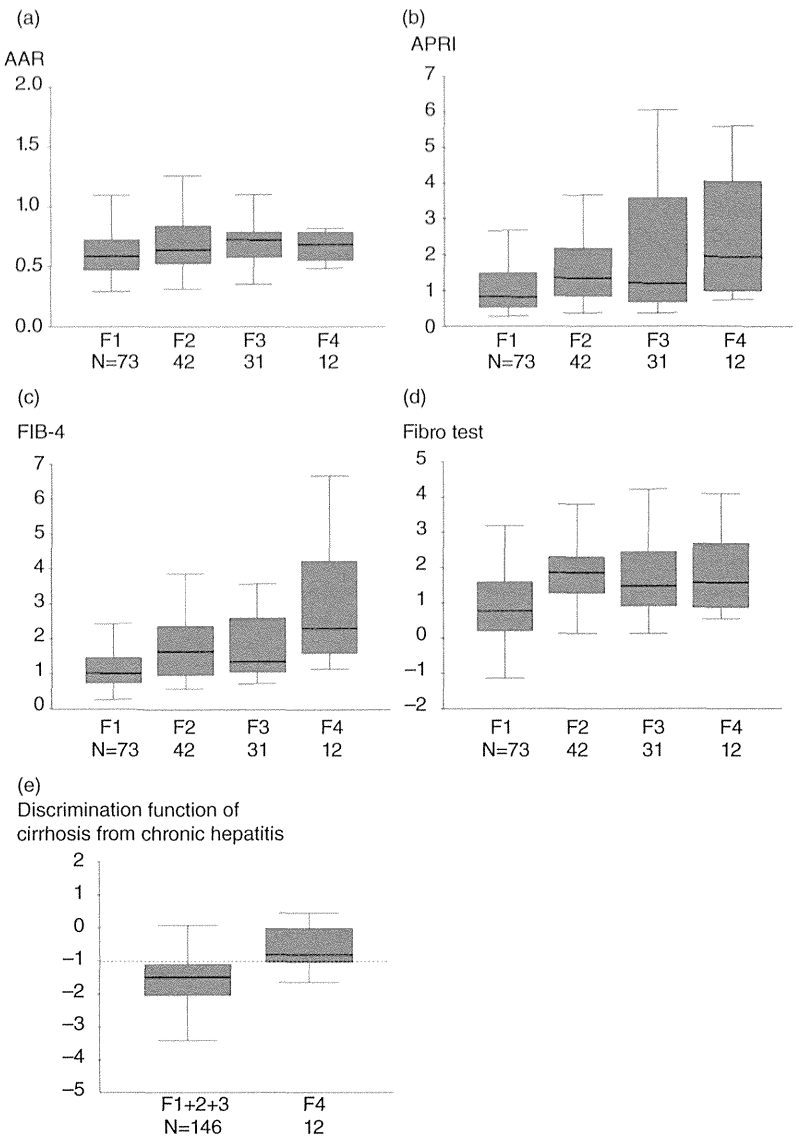

Figure 4 Previously published fibrosis scores. (a) Aspartate aminotransferase/alanine aminotransferase ratio (AAR),[19] (b) aspartate aminotransferase-to-platelet ratio index (APRI),[20] (c) FIB-4,[21] (d) FibroTest[22] and (e) discrimination function of cirrhosis from hepatitis in Japanese patients.[23]

the best management of patients with chronic hepatitis B. The FSB seems one of the ideal methods of approximating the fibrotic stage of chronic hepatitis B. Repeated measurement is quite suitable for patients with an unestablished treatment or trial, every 1 or 2 years, for example. Because the current regression function was generated from the data of HBV-related chronic liver disease, this equation would not be suitable for the recognition of hepatitis C virus-related chronic liver disease, alcoholic liver disease, and other congenital or autoimmune liver diseases. To recognize the latter diseases, other studies of individual diseases must be performed.

We compared the usefulness of the FSB with that of other fibrosis scores.[19–23] The more simple and less expensive AAR or APRI could not estimate fibrotic stages with poor correlation coefficients of 0.199 and 0.265, which are much lower than the coefficient of the FSB of 0.625. FibroTest, which contained three costly fibrosis markers (α-2-macroglobulin, haptoglobin and apolipo-

protein A1), also showed a low correlation coefficient of 0.330, suggesting that its usefulness was limited in HBV positive oriental patients. Although FIB-4 demonstrated the best coefficient of 0.412 among the fibrosis scores, significant overlaps were found between neighboring stages and obtained scores were not coordinated for real histological classification.

In conclusion, the FSB was a useful and reliable biomarker for prediction of liver fibrosis in patients with chronic HBV infection. The FSB is expected to be introduced and utilized in varied kinds of studies and trials. Its accuracy and reproducibility require further validation using higher numbers of patients in several countries other than Japan.

ACKNOWLEDGMENTS

THIS STUDY WAS proposed and initiated by Dr Shiro Iino and the project was performed with a grant from the Viral Hepatitis Research Foundation of Japan.

REFERENCES

1 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.
2 Myers RP, Tainturier MH, Ratziu V *et al.* Prediction of liver histological lesions with biochemical markers in patients with chronic hepatitis B. *J Hepatol* 2003; **39:** 222–30.
3 Hanna RF, Kased N, Kwan SW *et al.* Double-contrast MRI for accurate staging of hepatocellular carcinoma in patients with cirrhosis. *AJR Am J Roentgenol* 2008; **190:** 47–57.
4 Hagiwara M, Rusinek H, Lee VS *et al.* Advanced liver fibrosis: diagnosis with 3D whole-liver perfusion MR imaging–initial experience. *Radiology* 2008; **246:** 926–34.
5 Taouli B, Chouli M, Martin AJ, Qayyum A, Coakley FV, Vilgrain V. Chronic hepatitis: role of diffusion-weighted imaging and diffusion tensor imaging for the diagnosis of liver fibrosis and inflammation. *J Magn Reson Imaging* 2008; **28:** 89–95.
6 Montazeri G, Estakhri A, Mohamadnejad M *et al.* Serum hyaluronate as a non-invasive marker of hepatic fibrosis and inflammation in HBeAg-negative chronic hepatitis B. *BMC Gastroenterol* 2005; **5:** 32.
7 Zeng MD, Lu LG, Mao YM *et al.* Prediction of significant fibrosis in HBeAg-positive patients with chronic hepatitis B by a noninvasive model. *Hepatology* 2005; **42:** 1437–45.
8 Chrysanthos NV, Papatheodoridis GV, Savvas S *et al.* Aspartate aminotransferase to platelet ratio index for fibrosis evaluation in chronic viral hepatitis. *Eur J Gastroenterol Hepatol* 2006; **18:** 389–96.
9 Kim BK, Kim Y, Park JY *et al.* Validation of FIB-4 and comparison with other simple noninvasive indices for predicting liver fibrosis and cirrhosis in hepatitis B virus-infected patients. *Liver Int* 2010; **30:** 546–53.
10 Wu SD, Wang JY, Li L. Staging of liver fibrosis in chronic hepatitis B patients with a composite predictive model: a comparative study. *World J Gastroenterol* 2010; **16:** 501–7.
11 Sökücü S, Gökçe S, Güllüoğlu M, Aydoğan A, Celtik C, Durmaz O. The role of the non-invasive serum marker FibroTest-ActiTest in the prediction of histological stage of fibrosis and activity in children with naïve chronic hepatitis B infection. *Scand J Infect Dis* 2010; **42:** 699–703.
12 Liu HB, Zhou JP, Zhang Y, Lv XH, Wang W. Prediction on liver fibrosis using different APRI thresholds when patient age is a categorical marker in patients with chronic hepatitis B. *Clin Chim Acta* 2011; **412:** 33–7.
13 Park SH, Kim CH, Kim DJ *et al.* Usefulness of Multiple Biomarkers for the Prediction of Significant Fibrosis in Chronic Hepatitis B. *J Clin Gastroenterol* 2011; **45:** 361–5.
14 Engstrom-Laurent A, Loof L, Nyberg A, Schroder T. Increased serum levels of hyaluronate in liver disease. *Hepatology* 1985; **5:** 638–42.
15 Murawaki Y, Ikuta Y, Koda M, Kawasaki H. Serum type III procollagen peptide, type IV collagen 7S domain, central triple-helix of type IV collagen and tissue inhibitor of metalloproteinases in patients with chronic viral liver disease: relationship to liver histology. *Hepatology* 1994; **20:** 780–7.
16 Fabris C, Falleti E, Federico E, Toniutto P, Pirisi M. A comparison of four serum markers of fibrosis in the diagnosis of cirrhosis. *Ann Clin Biochem* 1997; **34:** 151–5.
17 Desmet VJ, Gerber M, Hoofnagle JH, Manns M, Sheuer PJ. Classification of chronic hepatitis: diagnosis, grading, and staging. *Hepatology* 1994; **19:** 1513–20.
18 IBM SPSS Inc. *IBM SPSS for Windows Version 19.0 Manual.* Armonk NY, USA: SPSS Japan Inc., an IBM company, 2009.
19 Sheth SG, Flamm SL, Gordon FD *et al.* AST/ALT ratio predicts cirrhosis in patients with chronic hepatitis C virus infection. *Am J Gastroenterol* 1998; **93:** 44–8.
20 Wai CT, Greenson JK, Fontana RJ *et al.* A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology* 2003; **38:** 518–26.
21 Sterling RK, Lissen E, Clumeck N *et al.* Develoment of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. *Hepatology* 2006; **43:** 1317–25.
22 Imbert-Bismut F, Ratziu V, Pieroni L *et al.* Biochemical imarkers of liver fibrosis in patients with hepatitis C virus infection: a prospective study. *Lancet* 2001; **357:** 1069–75.
23 Ikeda K, Saitoh S, Kobayashi M *et al.* Distinction between chronic hepatitis and liver cirrhosis in patients with hepatitis C virus infection. Practical Discriminant function using common laboratory data. *Hepatol Res* 2000; **18:** 252–66.
24 Lok ASF, McMahon BJ. AASLD Practice Guidelines. Chronic hepatitis B: update 2009. *Hepatology* 2009; **50:** 1–36.

J Gastroenterol (2012) 47:1006–1013
DOI 10.1007/s00535-012-0559-2

ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Changes in the serum level of hepatitis B virus (HBV) surface antigen over the natural course of HBV infection

Akihiro Matsumoto · Eiji Tanaka · Susumu Morita · Kaname Yoshizawa · Takeji Umemura · Satoru Joshita

Received: 13 October 2011 / Accepted: 30 January 2012 / Published online: 29 February 2012

Abstract
Background Despite its status as a potential biomarker of hepatitis B virus (HBV) response to interferon treatment, the changes in hepatitis B surface antigen (HBsAg) levels over the natural course of HBV carriers have not been analyzed sufficiently.
Methods A total of 101 HBV carriers were followed prospectively from 1999 to 2009. HBsAg level was measured yearly during the followed period.
Results HBsAg levels at baseline ranged from −1.4 to 5.32 log IU/ml, with a median value of 3.2 log IU/ml. Lower HBsAg levels were significantly associated with higher age and lower HBV replication status. The rate of change of HBsAg levels showed two peaks, with a cut-off value of −0.4 log IU/year. Based on this, patients were tentatively classified into rapid decrease (rate of change <−0.4 log IU/year) and non-rapid decrease groups. All baseline levels of HBsAg, HB core-related Ag, and HBV DNA were lower in the rapid decrease group than in the non-rapid decrease group. Patients with persistently positive HBeAg were all classified into the non-rapid decrease group. In patients with persistently negative HBeAg, HBV DNA levels were significantly ($P = 0.028$) lower in the rapid decrease group than in the non-rapid decrease group.
Conclusions Lower baseline HBsAg levels were significantly associated with older age and lower viral activity. Both a loss of HBeAg detection as well as inactive replication of HBV are suggested to be fundamental factors contributing to a rapid decrease in HBsAg over the natural course of HBV infection.

Keywords Hepatitis B virus · Hepatitis B surface antigen · Hepatitis B core-related antigen · Serum level · Natural course

Abbreviations

HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
IU	International unit
HBcrAg	Hepatitis B virus core-related antigen
HCC	Hepatocellular carcinoma
NA	Nucleos(t)ide analogue
HBeAg	Hepatitis B e antigen
CLEIA	Chemiluminescent enzyme immunoassay
Da	Dalton
HR	Hazard ratio
cccDNA	Covalently closed circular DNA

Introduction

With an estimated 350–400 million cases of chronic infection, hepatitis B virus (HBV) infection is a major worldwide health problem [1]. Chronic infection of HBV often leads to chronic hepatitis and eventually to liver cirrhosis and hepatocellular carcinoma [2, 3]. During infection, hepatitis B surface antigen (HBsAg), which is a component of the virion envelope, is secreted into the

A. Matsumoto · E. Tanaka (✉) · S. Morita · K. Yoshizawa · T. Umemura · S. Joshita
Department of Medicine, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto, Nagano 390-8621, Japan
e-mail: etanaka@shinshu-u.ac.jp

K. Yoshizawa
Department of Gastroenterology, National Hospital Organization Shinshu Ueda Medical Center, 1-27-21 Midorigaoka, Ueda 386-8610, Japan

bloodstream in large amounts as subviral particles. Thus, serum HBsAg is routinely used as a marker for detection of HBV infection.

Recently, several groups have reported that HBsAg levels can be used as an indicator of the response to peg-interferon in chronic hepatitis B similarly to the conventional markers of HBV DNA level and hepatitis B (HB) e antigen/antibody status [4, 5]. Since HBV carriers who clear HBsAg usually have a better prognosis than those who do not [6–8], it may be worthwhile to monitor HBsAg levels in the natural disease course of HBV infection. However, such changes need to be clarified more thoroughly to validate their clinical significance. In the present study, we analyzed the changes in HBsAg levels in a cohort of HBV carriers who were followed prospectively and compared them with those of HBV DNA and HB core-related antigens (HBcrAg) levels.

Patients and methods

Patients

A total of 101 HBV carriers were followed prospectively from 1999 to 2009. Patients were selected consecutively between 1997 and 1999 and met the following conditions: (1) HBsAg was positive in at least two examinations performed over 1 year apart; (2) no complications of hepatocellular carcinoma (HCC) or signs of hepatic dysfunction, such as jaundice or ascites, were observed; (3) nucleos(t)ide analogues (NAs) were not administered at the start of follow-up; and (4) patients were negative for hepatitis C and human immunodeficiency virus antibodies. The clinical and virological characteristics of our cohort are shown in Table 1. The 101 patients consisted of 57 men and 44 women with a median age of 50 years (range 15–83 years). Hepatitis B e antigen (HBeAg) was positive in 38 (38%) patients and negative in 63 (63%). Of the 38 patients with HBeAg, 15 remained positive and 23 became negative during the follow-up period. Alanine aminotransferase (ALT) level flares of over 1,000 IU/L were observed in four (17%) of the 23 patients with HBeAg loss, but in none of the 15 patients with persistent HBeAg ($P = 0.138$). HBV genotype distribution was A in three (3%) patients, B in nine (9%), C in 87 (86%), and undetermined in two (2%). All patients were seen at Shinshu University Hospital or one of its affiliated hospitals. Our cohort tended to have a higher prevalence of cirrhosis (19%) and HCC (14%). These tendencies may be attributed to the higher age distribution in our cohort than that in other cohorts of HBsAg studies [6, 9, 10].

Patients were seen at least once a year during the 10 years of follow-up. The presence of cirrhosis was judged by histological findings and/or typical findings seen in cirrhosis, such as esophageal varices and splenomegaly. Screening for HCC was done using ultrasonography (US), computed tomography (CT), and/or magnetic resonance (MR) imagining at least once a year. The presence of complicating HCC was judged by evidence of characteristic hepatic masses on liver CT, MRI, and/or hepatic angiography. Serum samples were collected on a yearly basis and immediately stored at −20°C or below until assayed. This study was approved by the Ethics Committee of Shinshu University.

Hepatitis B viral markers

Serological markers for HBV, including HBsAg, HBeAg, and HBe antibody, were tested using commercially

Table 1 Clinical and virological characteristics of patients with respect to HBeAg status

Characteristic	Overall ($n = 101$)	HBeAg-positive ($n = 38$)	HBeAg-negative ($n = 63$)	P
At baseline				
Age (years)[a]	50 (15 to 83)	42 (15 to 72)	53 (25 to 83)	<0.001
Male[b]	57 (56%)	22 (58%)	35 (56%)	>0.2
With cirrhosis[b]	19 (19%)	10 (26%)	9 (14%)	0.188
ALT (IU/L)[a]	31 (10 to 447)	47 (13 to 447)	29 (10 to 81)	0.002
HBV genotype (A:B:C:UD)	3:9:87:2	1:0:36:1	2:9:51:1	0.144
HBsAg (log IU/ml)[a]	3.2 (−1.4 to 5.3)	3.7 (1.6 to 5.3)	2.9 (−1.4 to 4.3)	<0.001
HBcrAg (log U/ml)[a]	3.8 (<3.0 to >6.8)	6.8 (<3.0 to >6.8)	3.1 (<3.0 to >6.8)	<0.001
HBV DNA (log copies/ml)[a]	4.7 (neg. to >9.5)	7.4 (2.4 to >9.5)	3.6 (neg. to 8.3)	<0.001
During follow-up				
Followed period (years)[a]	5 (1 to 10)	6 (1 to 10)	5 (1 to 10)	>0.2
Clearance of HBsAg[b]	20 (20%)	3 (8%)	17 (27%)	0.022
Complication of HCC[b]	14 (14%)	8 (21%)	6 (10%)	0.139
Introduction of NAs[b]	23 (23%)	11 (29%)	12 (19%)	>0.2

UD undetermined

[a] Data are expressed as median (range)

[b] Data are expressed as positive number (%)

available enzyme immunoassay kits (Abbott Japan Co., Ltd., Tokyo, Japan). Quantitative measurement of HBsAg was done using an HISCL® HBsAg assay based on the chemiluminescence enzyme immunoassay (CLEIA) (Sysmex Co. Ltd., Kobe, Japan), which had a quantitative range from −1.5 to 3.3 log IU/ml. End titer was determined by diluting samples with normal human serum when initial results exceeded the upper limit of the assay range. Changes in HBsAg levels during the natural course of HBV infection were calculated as: difference in HBsAg level at baseline and at last visit (not undergoing NA treatment) divided by the corresponding follow-up time. Results were expressed as log change per year. Points when patients were negative for HBsAg were omitted in calculations; thus, three patients who had cleared HBsAg by the first follow-up were excluded from the study. Changes in HBsAg levels during NA treatment were calculated similarly using the differences in HBsAg levels between the start and either the end of NA treatment or the last visit.

Serum HBcrAg levels were measured using a CLEIA-based HBcrAg assay kit with a fully automated Lumipulse System analyzer (Fujirebio Inc., Tokyo, Japan) as described previously [11]. Briefly, 150 μl of serum was incubated with 150 μl of pretreatment solution containing 15% sodium dodecyl sulphate at 60°C for 30 min. After heat treatment, 120 μl of pretreated specimen was added to a ferrite microparticle suspension in an assay cartridge. Ferrite particles were coated with a monoclonal antibody mixture (HB44, HB61, and HB114) against denatured HBcAg, HBeAg, and the 22 kDa precore protein. [12] After 10 min of incubation at 37°C and washing, further incubation was carried out for 10 min at 37°C with alkaline phosphatase conjugated with two kinds of monoclonal antibodies (HB91 and HB110) against denatured HBcAg, HBeAg, and the 22 kDa precore protein. After washing, 200 μl of substrate solution was added to the test cartridge, which was then incubated for 5 min at 37°C. The relative chemiluminescence intensity was measured, and HBcrAg concentration was calculated by a standard curve generated using recombinant pro-HBeAg (amino acids −10 to 183 of the precore/core gene product). The immunoreactivity of pro-HBeAg at 10 fg/ml was defined as 1 U/ml. HBcrAg was expressed in terms of log U/ml, and the quantitative range was set at 3.0–6.8 log U/ml.

Serum concentration of HBV DNA was determined using an AccuGene m-HBV kit (Abbott Japan Co., Ltd.) with a quantitative range of 1.7–9.5 log copies/ml when tested in a sample volume of 0.2 ml. Six HBV genotypes (A–F) were evaluated according to the restriction patterns of DNA fragments from the method reported by Mizokami et al. [13].

Statistical analyses

Correlations between variables were calculated using the Spearman correlation coefficient test. The Fisher's exact and Pearson's Chi-square tests were adopted to test for differences between subgroups of patients. To compare continuous data, the Mann–Whitney *U* test was employed. To compare paired continuous data, the Wilcoxon signed-rank test for matched pairs was used. The Kaplan–Meier method was used to estimate positive rates of HBsAg and the occurrence rate of HCC. Multivariate analyses were performed using the Cox regression model. Variables associated with a P value of <0.2 in univariate analyses were included in a stepwise Cox regression analysis to identify independent factors associated with clearance of HBsAg. All tests were performed using the IBM SPSS Statistics Desktop for Japan ver. 19.0 (IBM Japan Inc., Tokyo, Japan). *P* values of less than 0.05 were considered to be statistically significant.

Results

Follow-up of patients

Twenty three (23%) of the 101 patients enrolled dropped out of the study for reasons of changing addresses (11 patients) or halting hospital visits (12 patients). Among the remaining 78 patients, six died (four from HCC, one from hepatic failure, and one from old age) and one underwent liver transplantation due to hepatic failure. Thus, 71 patients completed the full follow-up period of 10 years.

Long term treatment with NAs, such lamivudine, was introduced in 23 patients (23%) during the study period.

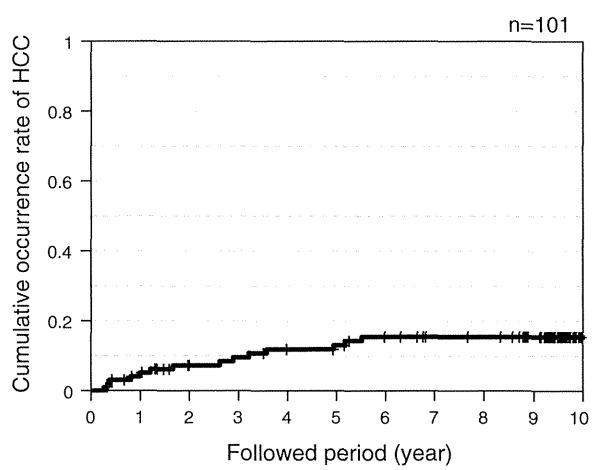

Fig. 1 Changes in the cumulative occurrence rate of HCC during the follow-up period

These patients commenced treatment after showing clinical and/or histological features of chronic active hepatitis B. The treatment period with NAs was excluded from our analysis of HBsAg level changes during the natural disease course.

Complicating HCC was seen in 14 patients within 6 years of their first visit (Fig. 1), leading to an annual rate of HCC occurrence of 2.3% per year for the first 6 years of follow-up. HCC was seen after the disappearance of HBsAg in a 90-year-old woman with negative HBeAg and HBV DNA at the time of diagnosis.

HBsAg levels at baseline and during clinical course

Baseline HBsAg levels ranged from −1.4 to 5.32 log IU/ml, with a median value of 3.2 log IU/ml (Fig. 2). Table 2 shows a comparison of clinical and virological characteristics between patients with lower and higher HBsAg levels divided at the median level. Older patients were significantly more prevalent in the lower level group than in the higher level group. Genotype B was only seen in the lower level group. Detection of HBeAg and median levels of both HBV DNA and HBcrAg were significantly lower in the lower level group. Clearance of HBsAg during the follow-up period was more frequent in the lower level group, but the occurrence of HCC was comparable between the two groups. Of the 52 patients with higher HBsAg levels at baseline, five lost HBsAg positivity and the remaining 47 did not. Median levels of HBV DNA (3.2 vs. 6.0 log copies/ml, $P = 0.023$), HBsAg (3.5 vs. 3.8 log IU/ml, $P = 0.091$), and HBcrAg (3.2 vs. 5.5 log U/ml, $P = 0.095$) tended to be lower in the former than in the latter group of patients at baseline, but the difference was statistically significant for HBV DNA level only. Median age (49 vs. 46 years, $P > 0.2$), male gender (80 vs. 45%, $P = 0.183$), and median ALT level (32 vs. 40 IU/L, $P > 0.2$) did not differ between the two groups at baseline.

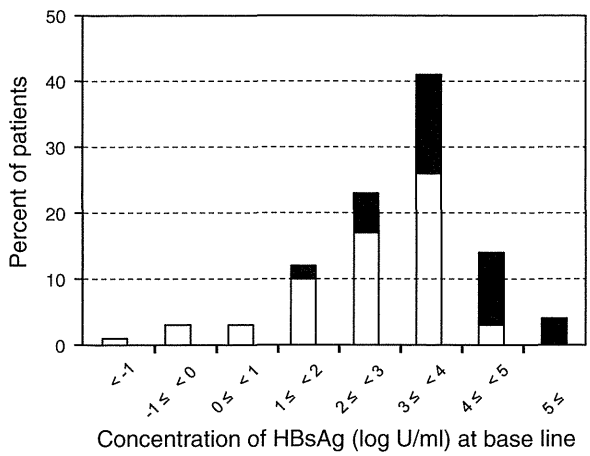

Fig. 2 Distribution of HBsAg concentration at baseline. *Closed bars* indicate patients with detectable HBeAg and *open bars* indicate those without

HBsAg levels at baseline were further analyzed according to age and HBeAg status, and the trend of HBsAg distribution was compared to those of HBcrAg and HBV DNA (Fig. 3). HBsAg levels in HBeAg-positive patients were distributed in a higher range, and the association of HBsAg with age was faint ($r = -0.291$, $P = 0.076$). On the other hand, HBsAg levels were distributed in a higher range in patients younger than 50 years of age, but were distributed more widely in patients 50 years or older. HBsAg levels in HBeAg-negative patients decreased significantly ($r = -0.453$, $P < 0.001$) with age. Furthermore, whereas HBcrAg levels in HBeAg-positive patients ($r = -0.260$, $P = 0.115$) were distributed in a higher range among all

Table 2 Comparison of clinical and virological characteristics between patients with serum HBsAg levels less than 3.2 log IU/ml and those with levels equal to or higher than 3.2 log IU/ml

Characteristic	HBsAg level at baseline		*P*
	<3.2 log IU/ml ($n = 49$)	≥3.2 log IU/ml ($n = 52$)	
At baseline			
Age (years)[a]	55 (32 to 83)	45 (15 to 72)	<0.001
Male[b]	32 (65%)	25 (48%)	0.108
With cirrhosis[b]	13 (27%)	6 (12%)	0.075
ALT (IU/L)[a]	28 (10 to 119)	39 (12 to 447)	0.089
HBV genotype (A:B:C:UD)	1:9:38:1	2:0:49:1	0.018
HBeAg[b]	11 (22%)	27 (52%)	0.004
HBcrAg (log U/ml)[a]	3.3 (<3.0 to >6.8)	5.5 (<3.0 to >6.8)	<0.001
HBV DNA (log copies/ml)[a]	3.7 (<1.7 to 8.3)	6.0 (neg. to >9.5)	0.001
During follow-up			
Followed period (years)[a]	4 (1 to 10)	8 (1 to 10)	0.001
Clearance of HBsAg[b]	15 (31%)	5 (10%)	0.012
Occurrence of HCC[b]	9 (18%)	5 (10%)	>0.2
Introduction of NAs[b]	9 (18%)	14 (27%)	>0.2

UD undetermined

[a] Data are expressed as median (range)

[b] Data are expressed as positive number (%)

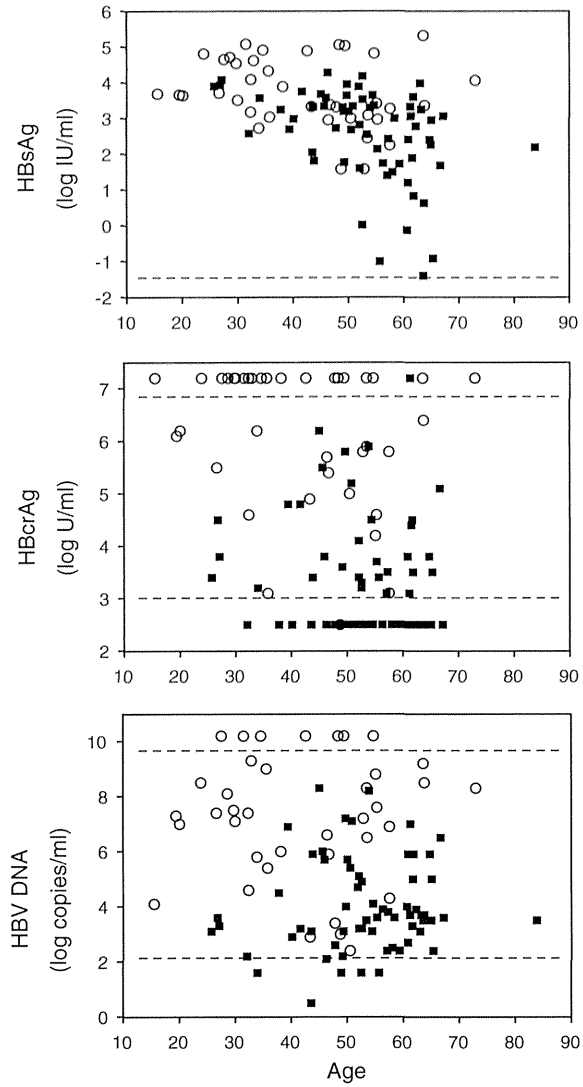

Fig. 3 HBsAg, HBcrAg, and HBV DNA levels analyzed according to HBeAg status and patient age. *Open circles* indicate patients with detectable HBeAg and *closed squares* indicate those without

ages, those in HBeAg-negative patients ($r = -0.103$, $P > 0.2$) were found in a lower range. A similar trend was seen for HBV DNA level distribution ($r = 0.015$, $P > 0.2$ and $r = 0.146$, $P > 0.2$, respectively).

Changes in HBsAg levels during the follow-up period

Positivity for HBsAg decreased gradually over the follow-up period (Fig. 4). A total of 20 patients cleared HBsAg during the follow-up period, for a disappearance rate of 2.1% per year. Clinical and virological backgrounds were compared between patients with and without clearance of HBsAg in Table 3. Patients losing HBsAg positivity were

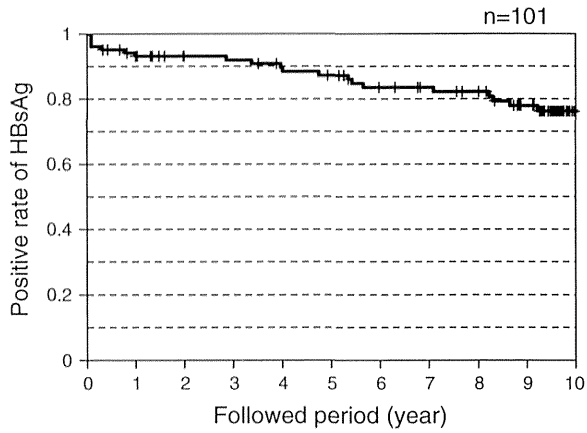

Fig. 4 Changes in HBsAg positivity during the follow-up period

significantly older than those who did not. Baseline levels of HBsAg, HBcrAg, and HBV DNA were significantly lower in these patients as well. Clearance of HBsAg was significantly associated with HBV DNA (HR 3.6, 95% CI 1.1–11.4, $P = 0.033$) and HBcrAg (HR 4.0, 95% CI 1.1–14.9, $P = 0.036$) levels at baseline by multivariate analysis. Of the 20 patients who cleared HBsAg, seven were positive for HBV DNA (range, positive 3.0 log copies/ml) and three were positive for HBcrAg (range 3.0–3.2 U/ml).

Figure 5 shows the distribution of patients according to the rate of change of HBsAg levels. Of the 98 patients analyzed, 79 (81%) showed a decrease in HBsAg. Although this level increased in 19% of patients, such changes were less than 0.2 log IU/year. The rate of change of HBsAg levels peaked at a cut-off value of −0.4 log IU/year. Accordingly, patients were tentatively classified into the rapid decrease group (rate of change <-0.4 log IU/year) and the non-rapid decrease group (rate of change ≥ -0.4 log IU/year). Median age, gender distribution, prevalence of cirrhosis, ALT level, and genotype distribution did not differ between the two groups (Table 4). Levels of HBsAg, HBcrAg, and HBV DNA were significantly lower in the rapid decrease group than in the non-rapid one. Whereas all patients with persistently positive HBeAg were classified into the non-rapid group, patients with persistently negative HBeAg fell more frequently into the rapid decrease group (77%) than into the non-rapid decrease group (54%). In those patients, HBV DNA levels were significantly ($P = 0.028$) lower in the rapid decrease group (median 3.4, range <2.1–5.9 log copies/ml) than in the non-rapid decrease group (median 3.8, range <2.1–8.1 log copies/ml). Complicating HCC was lower in the rapid decrease group, but this difference was not statistically significant.

The median change in HBsAg level before NA treatment (−0.117 log IU/ml/year; range −2.4 to 1.41 log

Table 3 Comparison of clinical and virological characteristics between patients with and without clearance of HBsAg

Characteristic	Clearance of HBsAg		*P*
	Positive ($n = 20$)	Negative ($n = 81$)	
At baseline			
Age (years)[a]	56 (30 to 65)	50 (16 to 84)	0.038
Male[b]	8 (40%)	36 (44%)	>0.2
With cirrhosis[b]	4 (20%)	15 (18%)	1.000
ALT (IU/L)[a]	26 (10 to 108)	35 (13 to 447)	0.057
HBV genotype (A:B:C:UD)	0:2:18:0	3:7:69:2	>0.2
HBeAg[b]	3 (15%)	35 (43%)	0.022
HBsAg (log IU/ml)[a]	1.7 (−1.7 to 4.2)	3.3 (0.83 to 5.3)	<0.001
HBcrAg (log U/ml)[a]	3.0 (3.0 to >6.8)	4.7 (3.0 to >6.8)	<0.001
HBV DNA (log copies/ml)[a]	3.0 (<1.7 to 7.6)	5.7 (neg. to >9.5)	<0.001
During follow-up			
Followed period (years)[a]	4.4 (0.31 to 10.0)	5.2 (0.1 to 10.0)	>0.2
Occurrence of HCC[b]	1 (5.0%)	13 (16.0%)	>0.2
Introduction of NAs[b]	0 (0%)	23 (28%)	0.006

UD undetermined

[a] Data are expressed as median (range)

[b] Data are expressed as positive number (%)

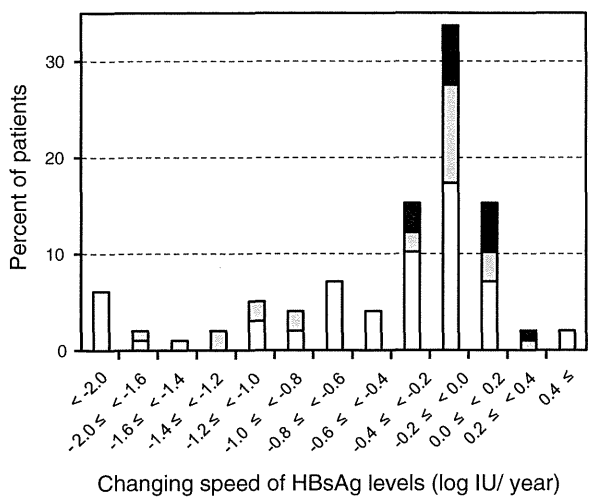

Fig. 5 Distribution of patients classified according to rate of change of HBsAg levels (log IU/year) during follow-up period. *Closed bars* indicate patients with persistent HBeAg-positive status. *Shaded bars* indicate patients who became negative for HBeAg during follow-up period. *Open bars* indicate patients with persistent HBeAg-negative status

IU/ml/year) was similar ($P > 0.2$) to that after starting NA treatment (−0.017 log IU/ml/year; range −5.18 to 0.17 log IU/ml/year) in the 20 patients who commenced therapy with NAs during the study period.

Discussion

During the natural course of HBV infection, HBsAg levels showed almost normal distribution, making a sharp peak at a median value of 3.2 log IU/ml. Lower HBsAg levels were significantly associated with older age and lower viral activity, but not with gender or genotype. A similar trend was observed in patients who cleared HBsAg in our cohort. Chan et al. [10] reported that HBsAg levels were significantly lower in HBeAg-negative patients than in HBeAg-positive ones and tended to fall in accordance with decreases in HBV DNA levels. Simonetti et al. [6] reported that clearance of HBsAg was associated with older age, but not with gender or genotype, in a prospective population-based cohort study. Chu et al. [9] also reported that HBsAg clearance was associated with older age, in which the cumulative probability of clearance increased disproportionally with a longer follow-up period. In light of these results as well as of our own, it appears that lower HBsAg levels are closely associated with older age and lower activity of HBV replication. The HBsAg clearance rate of 2.1% per year in the current study was three times higher than that of the 0.7% per year reported by Simonetti et al. [6]. However, the median age at the start of their follow-up (20 years) was considerably lower than that in our report (50 years). Chu et al. [9] followed 1965 asymptomatic HBV carriers that were positive for HBe antibodies in whom the mean age at baseline was 35.6 years, revealing a HBsAg clearance rate of 0.8% per year after 10 years of follow-up that increased to 1.8% per year over a 25-year observation period. HBsAg clearance appeared to increase as patients aged in that cohort, which may at least partly explain the higher clearance rate found in the present study.

Because HBsAg level is closely associated with age, we analyzed this relationship and compared it with those of HBcrAg and HBV DNA. HBsAg levels decreased in association with age in HBeAg-negative patients. A similar but faint association was also seen in HBeAg-positive patients. On the other hand, HBcrAg and HBV DNA levels

Table 4 Comparison of clinical and virological characteristics between patients with rapid and non-rapid decrease of HBsAg

Characteristic	Rapid decrease ($n = 31$)	Non-rapid decrease ($n = 67$)	P
At baseline			
Age (years)[a]	52 (15 to 65)	49 (19 to 83)	0.338
Male[b]	17 (55%)	38 (57%)	1.000
With cirrhosis[b]	6 (19%)	13 (19%)	1.000
ALT (IU/L)[a]	27 (10 to 108)	36 (13 to 447)	0.230
HBV genotype (A:B:C:UD)	1:4:26:0	2:4:59:2	0.617
HBeAg-positive[b]	7 (23%)	31 (46%)	0.028
HBsAg (log IU/ml)[a]	2.8 (−1.0 to 5.0)	3.3 (0.8 to 5.3)	0.001
HBcrAg (log U/ml)[a]	<3.0 (<3.0 to >6.8)	5.1 (<3.0 to >6.8)	<0.001
HBV DNA (log copies/ml)[a]	3.7 (<1.7 to >9.5)	5.9 (neg. to >9.5)	0.002
During follow-up			
Followed period (years)[a]	3 (1 to 9)	6 (1 to 10)	<0.001
Change in HBeAg status			0.012
Persistent positive[b]	0 (0%)	15 (22%)	
Became negative[b]	7 (23%)	16 (24%)	
Persistent negative[b]	24 (77%)	36 (54%)	
Clearance of HBsAg[b]	18 (58%)	0 (0%)	<0.001
Complication of HCC[b]	2 (7%)	12 (18%)	0.214
Introduction of NAs[b]	4 (13%)	19 (28%)	0.125

UD undetermined

[a] Data are expressed as median (range)

[b] Data are expressed as positive number (%)

were more uniformly distributed with age in both HBeAg-positive and -negative patients. Therefore, it can be inferred that HBsAg level is affected by age in the natural course of HBV, even when the factor of viral activity is excluded. The precise mechanism of this trend is at present unclear, but may be attributed to the character of HBsAg itself, and not to that of HBV antigens, because HBcrAg levels showed a similar trend as HBV DNA levels. Chan et al. [10] reported that a stronger correlation between HBV DNA and HBsAg was found in the HBeAg-positive phase than in the HBeAg-negative phase. This observation was clearly confirmed by our results in that the distribution pattern analyzed by age was similar between HBsAg and HBV DNA levels in HBeAg-positive patients but differed in HBeAg-negative ones.

The rate of change of HBsAg in the present study suggested the existence of two groups centered around a value of −0.4 log IU/year. A necessary decline in HBV replication was evident in the rapid decrease group, whose median HBV DNA level was lower than the 4.0 log copy/ml usually seen in inactive carriers of HBV. Since no patient with persistently positive HBeAg was classified into the rapid increase group, we presume that a loss of HBeAg is essential for a rapid decrease in HBsAg. In patients with persistently negative HBeAg, HBV DNA levels were significantly lower in the rapid decrease group than in the non-rapid decrease group. Therefore, not only a loss of HBeAg, but also a decline in HBV replication, appears to be fundamental factors necessary for a rapid decrease in HBsAg. Chan et al. [10] concluded that HBs antigen level remained stable in HBe antigen-positive patients and reduced slowly in HBe antigen-negative patients. Our results are similar, but further imply that a decline in HBV replication is also required. The rate of HBsAg level decrease was similar before and after starting NA treatment in the present study. However, additional studies in larger cohorts will be required to determine this particular relationship.

We analyzed HBcrAg in addition to HBsAg as an HBV-related antigen in the present study to further clarify the characteristics of HBsAg. The HBcrAg assay measures serum levels of HBcAg, HBeAg, and the 22 kDa precore protein [12] simultaneously using monoclonal antibodies that recognize the common epitopes of these denatured antigens. Since the assay measures all antigens transcribed from the pre-core/core gene, it is regarded as core-related [14]. It is possible that levels of HBsAg and HBcrAg have different properties because transcriptions of these two antigens are regulated by alternative enhancer-promoter systems in the HBV genome [15]. Recent studies have shown that HBsAg quantification may represent a surrogate marker of cccDNA concentration in the liver and a potential tool to monitor virologic response to interferon treatment [4, 5, 16]. On the other hand, serum HBcrAg has been reported to accurately reflect intracellular levels of HBV cccDNA even during nucleos(t)ide treatment [11, 17, 18], and was found to be useful for identifying patients who were likely to show relapse of hepatitis after the discontinuation of NAs [19, 20] or who had a higher possibility to develop hepatocellular carcinoma even under NA treatment [17]. Our results here suggest that there exists a

difference in natural course changes between HBsAg and HBcrAg levels. We recently reported that the combined use of these two antigens was useful for predicting the occurrence of hepatitis relapse after cessation of NAs [21]. Such results also indicated that levels of HBsAg and HBcrAg had different clinical significance despite the fact that both antigen levels are generally considered to reflect the amount of HBV cccDNA in hepatocytes.

Complicating HCC occurred during the first 6 years of follow-up in our study at an annual occurrence rate of 2.3% per year for that period. This complication was seen at similar frequencies in patients with high and low baseline HBsAg levels as well as in patients who showed rapid and non-rapid decreases in HBsAg. Patients with lower HBsAg levels and those with rapid decreases in HBsAg have been shown to have lower levels of HBV replication, which would indicate a lower risk of complicating HCC. However, such patients also tend to be older and presumably more predisposed to HCC. The similar occurrence of HCC irrespective of HBsAg status may be attributed to the existence of these two contrary factors. Yuen et al. [7] reported that the risk of HCC in patients with HBsAg seroclearance was higher in those older than 50 years of age; indeed, the single patient who developed HCC after HBsAg seroclearance in the present study was a 90 year-old woman.

In conclusion, lower HBsAg levels were significantly associated with older age and lower viral activity, but not with gender or genotype. Both a loss of HBeAg positivity and a decline in HBV replication are suggested to be fundamental factors necessary for a rapid decrease in HBsAg. Furthermore, the clinical significance of HBsAg may be different from that of HBcrAg with regard to age. Future studies are required to clarify the difference between the two antigens.

Acknowledgments This study was supported in part by a research grant on hepatitis from the Japanese Ministry of Health, Labor, and Welfare of Japan. We thank Ms. Hiroe Banno for her secretarial assistance and thank Ms. Nozomi Kamijo and Ms. Etsuko Iigahama for their technical assistance. We also thank Mr. Trevor Ralph for his English editorial assistance.

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

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. 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.
3. Lok AS, McMahon BJ. Chronic hepatitis B. Hepatology. 2007;45:507–39.
4. Brunetto MR, Moriconi F, Bonino F, Lau GK, Farci P, Yurdaydin C, et al. Hepatitis B virus surface antigen levels: a guide to sustained response to peginterferon alfa-2a in HBeAg-negative chronic hepatitis B. Hepatology. 2009;49:1141–50.
5. Moucari R, Mackiewicz V, Lada O, Ripault MP, Castelnau C, Martinot-Peignoux M, et al. Early serum HBsAg drop: a strong predictor of sustained virological response to pegylated interferon alfa-2a in HBeAg-negative patients. Hepatology. 2009;49:1151–7.
6. Simonetti J, Bulkow L, McMahon BJ, Homan C, Snowball M, Negus S, et al. Clearance of hepatitis B surface antigen and risk of hepatocellular carcinoma in a cohort chronically infected with hepatitis B virus. Hepatology. 2010;51:1531–7.
7. Yuen MF, Wong DK, Fung J, Ip P, But D, Hung I, et al. HBsAg Seroclearance in chronic hepatitis B in Asian patients: replicative level and risk of hepatocellular carcinoma. Gastroenterology. 2008;135:1192–9.
8. Tseng TC, Kao JH. HBsAg seroclearance: the more and earlier, the better. Gastroenterology. 2009;136:1842–3. author reply 3–4.
9. Chu CM, Liaw YF. HBsAg seroclearance in asymptomatic carriers of high endemic areas: appreciably high rates during a long-term follow-up. Hepatology. 2007;45:1187–92.
10. Chan HL, Wong VW, Wong GL, Tse CH, Chan HY, Sung JJ. A longitudinal study on the natural history of serum hepatitis B surface antigen changes in chronic hepatitis B. Hepatology. 2010;52:1232–41.
11. Suzuki F, Miyakoshi H, Kobayashi M, Kumada H. Correlation between serum hepatitis B virus core-related antigen and intrahepatic covalently closed circular DNA in chronic hepatitis B patients. J Med Virol. 2009;81:27–33.
12. Kimura T, Ohno N, Terada N, Rokuhara A, Matsumoto A, Yagi S, et al. Hepatitis B virus DNA-negative dane particles lack core protein but contain a 22-kDa precore protein without C-terminal arginine-rich domain. J Biol Chem. 2005;280:21713–9.
13. Mizokami M, Nakano T, Orito E, Tanaka Y, Sakugawa H, Mukaide M, et al. Hepatitis B virus genotype assignment using restriction fragment length polymorphism patterns. FEBS Lett. 1999;450:66–71.
14. Tanaka E, Matsumoto A, Yoshizawa K, Maki N. Hepatitis B core-related antigen assay is useful for monitoring the antiviral effects of nucleoside analogue therapy. Intervirology. 2008;51(Suppl 1):3–6.
15. Lee WM. Hepatitis B virus infection. N Engl J Med. 1997;337:1733–45.
16. Chan HL, Wong VW, Tse AM, Tse CH, Chim AM, Chan HY, et al. Serum hepatitis B surface antigen quantitation can reflect hepatitis B virus in the liver and predict treatment response. Clin Gastroenterol Hepatol. 2007;5:1462–8.
17. Hosaka T, Suzuki F, Kobayashi M, Hirakawa M, Kawamura Y, Yatsuji H, et al. HBcrAg is a predictor of post-treatment recurrence of hepatocellular carcinoma during antiviral therapy. Liver Int. 2010;30:1461–70.
18. Wong DK, Tanaka Y, Lai CL, Mizokami M, Fung J, Yuen MF. Hepatitis B virus core-related antigens as markers for monitoring chronic hepatitis B infection. J Clin Microbiol. 2007;45:3942–7.
19. Matsumoto A, Tanaka E, Minami M, Okanoue T, Yatsuhashi H, Nagaoka S, et al. Low serum level of hepatitis B core-related antigen indicates unlikely reactivation of hepatitis after cessation of lamivudine therapy. Hepatol Res. 2007;37:661–6.
20. Shinkai N, Tanaka Y, Orito E, Ito K, Ohno T, Hirashima N, et al. Measurement of hepatitis B virus core-related antigen as predicting factor for relapse after cessation of lamivudine therapy for chronic hepatitis B virus infection. Hepatol Res. 2006;36:272–6.
21. Matsumoto A, Tanaka E, Suzuki Y, Kobayashi M, Tanaka Y, Shinkai N, et al. Combination of hepatitis B viral antigens and DNA for prediction of relapse after discontinuation of nucleos(t)ide analogues in patients with chronic hepatitis B. Hepatol Res. 2012;42:139–49.

Hepatology Research

Hepatology Research 2012; 42: 139–149 doi: 10.1111/j.1872-034X.2011.00910.x

Original Article

Combination of hepatitis B viral antigens and DNA for prediction of relapse after discontinuation of nucleos(t)ide analogs in patients with chronic hepatitis B

Akihiro Matsumoto,[1] Eiji Tanaka,[1] Yoshiyuki Suzuki,[2] Mariko Kobayashi,[2] Yasuhito Tanaka,[4] Noboru Shinkai,[4] Shuhei Hige,[6] Hiroshi Yatsuhashi,[8] Shinya Nagaoka,[8] Kazuaki Chayama,[9] Masataka Tsuge,[9] Osamu Yokosuka,[10] Fumio Imazeki,[10] Shuhei Nishiguchi,[11] Masaki Saito,[11] Kei Fujiwara,[5] Nobuyuki Torii,[3] Naoki Hiramatsu,[12] Yoshiyasu Karino[7] and Hiromitsu Kumada[2]

[1]*Department of Medicine, Shinshu University School of Medicine, Matsumoto,* [2]*Department of Hepatology, Toranomon Hospital,* [3]*Department of Internal Medicine and Gastroenterology, Tokyo Women's Medical University, Tokyo,* [4]*Department of Virology and Liver Unit, Nagoya City University Graduate School of Medical Sciences,* [5]*Gastroenterology Section, Nagoya Daini Red Cross Hospital, Nagoya,* [6]*Department of Gastroenterology and Hepatology, Graduate School of Medicine, Hokkaido University,* [7]*Department of Gastroenterology, Sapporo Kosei General Hospital, Sapporo,* [8]*The Clinical Research Center, NHO Nagasaki Medical Center, Omura,* [9]*Program for Biomedical Research, Division of Frontier Medical Science, Department of Medicine and Molecular Science, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima,* [10]*Department of Medicine and Clinical Oncology, Graduate School of Medicine, Chiba University, Chiba,* [11]*Division of Hepatobiliary and Pancreatic Diseases, Department of Internal Medicine, Hyogo College of Medicine, Hyogo, and* [12]*Department of Gastroenterology and Hepatology, Osaka University Graduate School of Medicine, Osaka, Japan*

Aim: The factors associated with hepatitis recurrence after discontinuation of nucleos(t)ide analogs (NAs) in patients with chronic hepatitis B were analyzed to predict the risk of relapse more accurately.

Methods: A total of 126 patients who discontinued NA therapy were recruited retrospectively. The clinical conditions of a successful discontinuation were set as alanine aminotransferase (ALT) below 30 IU/L and serum hepatitis B virus (HBV) DNA below 4.0 log copies/mL.

Results: Relapse of hepatitis B were judged to occur when maximal serum ALT became higher than 79 IU/L or when maximal serum HBV DNA surpassed 5.7 log copies/mL following NA discontinuation since these values corresponded with mean values of ALT (30 IU/L) and HBV DNA (4.0 log copies/mL), respectively. At least 90% of patients with either detectable hepatitis B e antigen or serum HBV DNA higher than 3.0 log copies/mL at the time of NA discontinuation relapsed within one year. In the remaining patients, higher levels of both hepatitis B surface and core-related antigens at the time of discontinuation, as well as a shorter course of NA treatment, were significantly associated with relapse by multivariate analysis.

Conclusions: It appears that negative results for hepatitis B e antigen and serum HBV DNA lower than 3.0 log copies/mL are essential for successful NA discontinuation, which may be attained by a longer treatment period. Levels of hepatitis B surface and core-related antigens are also significant factors independently associated with relapse of hepatitis.

Key words: discontinuation, hepatitis B core-related antigen, hepatitis B surface antigen, nucleos(t)ide analogs, relapse of hepatitis

Correspondence: Professor Eiji Tanaka, Department of Medicine, Gastroenterology Division, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto, Nagano 390-8621, Japan. Email: etanaka@shinshu-u.ac.jp

Financial support

This research was supported in part by a research grant from the Ministry of Health, Labor and Welfare of Japan.

Received 7 August 2011; revision 31 August 2011; accepted 5 September 2011.

INTRODUCTION

HEPATITIS B VIRUS (HBV) infection is a major health concern that has an estimated 350 to 400 million carriers worldwide. Chronic infection with HBV can cause chronic hepatitis, and may eventually develop into liver cirrhosis and hepatocellular carcinoma.[1–3] Over the last decade, major advances in the treatment of chronic hepatitis B have been made with nucleos(t)ide

analogs (NAs) such as lamivudine (LVD), adefovir dipivoxil (ADV), and entecavir (ETV).[4] NAs are orally administered and are associated with low rates of adverse effects. Treatment with NAs shows strong suppression of HBV replication and consequently rapid improvement of elevated ALT levels. Furthermore, these drugs have been reported to lower the risk of complicating cirrhosis and hepatocellular carcinoma,[5–7] and so NAs are becoming widely used to treat patients with chronic hepatitis B. On the other hand, NAs carry the risk of developing drug-resistance;[8] drug-resistant viruses emerging during treatment may be associated with hepatitis flare-ups. Hepatitis B patients are also required to undergo prolonged treatment with NAs because early discontinuance often leads to relapse of hepatitis and ensuing hepatic failure following rises in alanine aminotransferase (ALT) level.[9,10]

Serum HBV DNA is normally used to monitor the antiviral effect of NAs. HBV DNA decreases rapidly and becomes undetectable in the majority of patients who are treated with NAs,[11–13] but relapse after discontinuation is not rare.[14–17] Since it is also true that favorable virological and biochemical responses to NAs may continue indefinitely in some patients,[9,15] reliable markers that can predict relapse of hepatitis after NA discontinuation are needed. Such markers would benefit not only patients who are considering discontinuation of NA treatment, but also clinicians, hospitals, and the medical economy.

In the present study, we assessed several factors associated with relapse of hepatitis after discontinuation of NAs in patients with chronic hepatitis B, including hepatitis B viral antigens, which have been reported as new and promising markers for monitoring the effect of antiviral agents, such as interferon and NAs.

METHODS

Patients

A TOTAL OF 126 patients with chronic hepatitis B who underwent and completed NA treatment between 2000 and 2010 were enrolled in this study. Patients were recruited retrospectively from 11 hospitals across Japan (Toranomon Hospital, Hokkaido University Hospital, Nagoya City University Hospital, Shinshu University Hospital, Hiroshima University Hospital, National Hospital Organization Nagasaki Medical Center, Chiba University Hospital, The Hospital of Hyogo College of Medicine, Japanese Red Cross Nagoya Daini Hospital, and Tokyo Women's Medical University Hospital, Sapporo Kosei General Hospital) and met the following conditions: (i) serum ALT higher than 30 IU/L and serum HBV DNA higher than 4.0 log copies/mL were observed at least twice within the 6 months prior to administration of NAs; (ii) stored serum samples at initiation and discontinuation of NAs were available for measurements of viral markers; (iii) clinical outcomes were followed for at least 6 months after the discontinuation of NAs; and (iv) tests for hepatitis C and human immunodeficiency virus antibodies were negative. Hepatitis B surface antigen (HBsAg) was confirmed to be positive on at least two occasions at least 6 months apart in all patients before treatment. Patients complicated with hepatocellular carcinoma or signs of hepatic failure at treatment discontinuation were excluded from the study. Our cohort consisted of 83 men and 43 women with a median age of 46 (range, 19 to 79) years when NA administration was discontinued. Hepatitis B e antigen (HBeAg) was positive in 64 patients (51%) at the initiation of treatment and in 24 patients (19%) at its discontinuation. HBV genotype was A in two (2%) patients, B in five (4%), C in 102 (81%), and undetermined in 17 (13%). Thirty-five of the 126 patients in this study were younger than 35 years old. Although not recommended as the first line treatment for this group by Japanese guidelines,[18] NA treatment was commenced since chronic active hepatitis had been persisting in all cases irrespective of their HBeAg status (26 positive and nine negative) at the initiation of treatment.

The decision to discontinue NAs was made by individual physicians using similar, but not uniform, conditions. Four patients who halted NAs for financial reasons were included. No patient underwent interferon treatment during or after NA treatment. The decision to recommence NA administration was also made by individual physicians, essentially when relapse of hepatitis became obvious. With few exceptions, patients were seen at least once a month during the first year after discontinuation of NAs, and at least once every several months afterwards. Stored serum samples were kept frozen at −20°C or below until assayed. This study was approved by the Ethics Committees of all participating institutions.

Hepatitis B viral markers

Serological markers for HBV, including HBsAg, HBeAg, and antibody to HBe (anti-HBe) were tested using commercially available enzyme immunoassay kits (Abbott Japan Co., Ltd, Tokyo, Japan; Fujirebio Inc., Tokyo, Japan; and/or Sysmex Co., Kobe, Japan) at each hospital. Quantitative measurement of HBsAg[19] was done using a chemiluminescence enzyme immunoassay