Table 1 Demography and laboratory data of 305 patients in training group

	F1 ($n=172$)	F2 ($n=80$)	F3 ($n=37$)	F4 ($n=16$)
Demography				
Males : females	97:75	38:42	20:17	6:10
Age (median, range)	51 (22–69)	55 (29–68)	55 (27–69)	56.5 (29–65)
Laboratory data (median, range)				
WBC ($\times10^3$/mm^3)	4.7 (2.0–10.1)	4.3 (2.3–8.5)	4.5 (2.9–6.8)	4.7 (3.3–6.9)
Hemoglobin(g/dL)	14.6 (11.0–18.2)	14.4 (9.3–17.4)	14.6 (11.5–17.7)	14.55 (12.1–16.5)
Platelet ($\times10^3$/mm^3)	183 (52–364)	161 (82–387)	131 (74–237)	124 (7.7–191)
Albumin (g/dL)	4.1 (2.3–4.9)	4.0 (3.5–4.6)	3.9 (3.1–4.6)	3.8 (3.3–4.3)
Bilirubin (mg/dL)	0.8 (0.2–1.9)	0.7 (0.3–1.7)	0.9 (0.4–7.5)	0.8 (0.5–7.4)
AST (IU/L)	42 (16–386)	61 (16–332)	63 (13–238)	71 (30–160)
ALT (IU/L)	60.5 (12–1664)	84.5 (10–647)	108 (27–415)	90.5 (36–264)
γ-GTP (IU/L)	40 (7–383)	48 (10–262)	54 (13–209)	58 (21–195)
γ-Globulin (g/dL)	1.47 (0.58–3.40)	1.61 (1.02–2.41)	1.69 (0.66–2.64)	1.79 (1.22–2.73)
γ-Globulin (%)	19.4 (10.0–40.5)	20.9 (14.0–28.3)	21.3 (8.1–30.4)	22.7 (16.5–36.9)
α2-Macroglobulin (mg/dL)	269 (123–505)	335 (154–551)	369 (183–627)	317 (207–511)
Haptoglobin (mg/dL)	94.5 (<5–265)	75.5 (<5–263)	56 (<5–2031)	75 (30–142)
Apolipoprotein A1 (mg/dL)	132 (71–209)	131 (73–207)	124 (98–166)	121 (83–153)
Hyaluronic acid (μg/L)	25 (<5–407)	41.5 (<5–263)	71 (<5–326)	89.5 (5–246)
TIMP-1 (ng/mL)	165 (73–291)	173 (97–302)	182 (126–308)	192.5 (128–260)
TIMP-2 (ng/mL)	77.5 (31–210)	80 (34–307)	76 (46–143)	78 (58–110)
Procollagen III peptide (U/mL)	0.75 (0.47–1.50)	0.805 (0.61–1.70)	0.86 (0.53–1.50)	1.05 (0.66–1.60)
Type IV collagen 7S (ng/mL)	4.0 (1.7–73)	4.3 (2.1–11.0)	5.2 (3.2–11.0)	5.8 (4.3–9.4)

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

fibrotic stage consisted of only 11 patients and the score 3.58 was regarded as a rather low value, the scores of other stages of fibrosis were concordant with histological fibrosis.

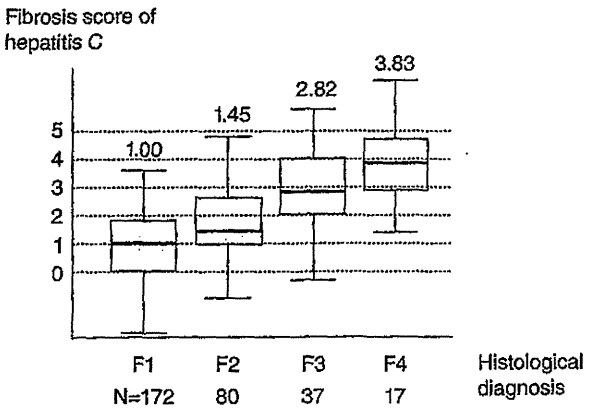

Figure 1 Box and whisker plots of fibrotic score of each group of histological fibrosis in the training dataset. Fibrotic score of hepatitis C (FSC) was generated by the function, $z = 2.89 \times ln$ (type IV collagen 7S) (ng/mL) $- 0.011 \times$ (platelet count) ($\times10^3$/mm^3) $+ 0.79 \times ln$ (total bilirubin) (mg/dL) $+ 0.39 \times ln$ (hyaluronic acid) (μg/L) $- 1.87$.

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

In order to evaluate the efficacy and usefulness of the obtained FSC, we compared with previously reported fibrotic scores using training data. AAR, APRI, FIB-4 and FibroTest showed only slight correlation with actual histological stage. APRI and FIB-4 demonstrated increasing trends of the score associated with histological fibrosis, but significant overlapping scores were found through F1 to F4. Spearman's correlation coefficients of AAR, APRI, FIB-4 and FibroTest were 0.021 ($P = 0.707$), 0.462 ($P < 0.001$), 0.440 ($P < 0.001$) and 0.415 ($P < 0.001$), respectively. Our FSC showed Spearman's correlation coefficient of 0.572 ($P < 0.001$), and was of much higher value than the others.

DISCUSSION

RECOGNITION OF SEVERITY of chronic hepatitis is essential in managing patients with chronic HCV infection: estimation of length of infection, existence of any previous hepatitis activity, presumption of current fibrotic stage, and prediction of future fibrotic progression and hepatocarcinogenesis. Differential diagnosis of cirrhosis from chronic hepatitis is especially important

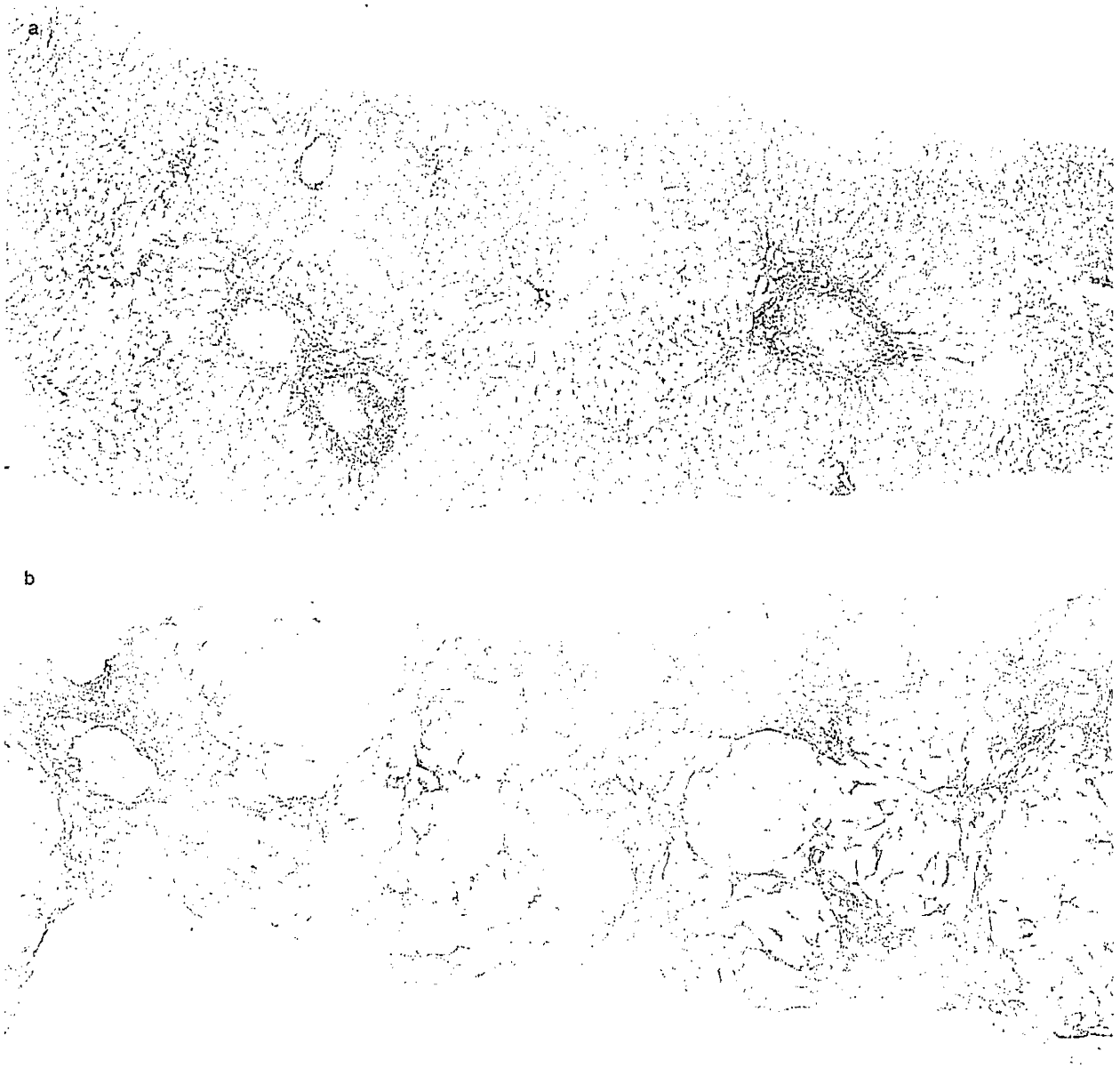

Figure 2 Case presentations of the training set. (a) A 55-year-old man with F1 fibrosis. Final regression function provided his fibrotic score as 1.16. (b) A 43-year-old man with F3 fibrosis with severe hepatitis activity. His regression coefficient was calculated as 4.98 (silver stain, ×40).

in the evaluation of chronic HCV infection. Identification of liver cirrhosis often leads to an important change in management of the patients: needs for fiberscopic examination for esophageal varices, ultrasonographic exploration for the association of liver cancer, and prediction of hepatic decompensation.

Recently, non-invasive estimation of severity of liver fibrosis has been reported in patients with HCV-related chronic hepatitis.[6–14] 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 discriminative functions were insufficient to

Table 2 Demography and laboratory data of 276 patients in validation group

	F1 ($n=156$)	F2 ($n=73$)	F3 ($n=36$)	F4 ($n=11$)
Demography				
Males : females	83:73	42:31	13:23	6:5
Age (median, range)	55 (15–74)	58 (32–77)	62.5 (30–78)	51 (38–73)
Laboratory data (median, range)				
WBC ($\times10^3$/mm^3)	5.1 (2.1–10.5)	4.8 (2.6–9.0)	4.85 (2.3–14.2)	3.9 (3.2–6.0)
Hemoglobin (g/dL)	14.2 (8.9–17.7)	14.4 (11.8–17.4)	14.1 (10.1–16.4)	13.6 (8.9–16.3)
Platelet ($\times10^3$/mm^3)	183 (59–440)	153 (80–265)	136 (64–348)	135 (79–153)
Albumin (g/dL)	4.3 (3.1–5.3)	4.3 (3.3–5.2)	4.05 (3.0–5.5)	3.9 (3.0–4.7)
Bilirubin (mg/dL)	0.7 (0.2–8.7)	0.7 (0.2–1.7)	0.8 (0.2–2.5)	0.8 (0.4–11.0)
AST (IU/L)	35 (11–1390)	49 (19–183)	80 (20–190)	96 (29–257)
ALT (IU/L)	49 (11–1635)	62 (12–575)	84 (14–218)	115 (29–303)
γ-GTP (IU/L)	35 (11–600)	52 (10–497)	51 (14–236)	112 (17–312)
γ-Globulin (g/dL)	1.47 (0.70–2.14)	1.60 (0.80–2.37)	1.71 (0.63–2.62)	2.19 (1.70–2.82)
γ-Globulin (%)	19.5 (9.2–26.4)	20.8 (10.8–30.8)	22.4 (9.5–29.9)	27.4 (21.8–35.3)
α2-Macroglobulin (mg/dL)	271.5 (126–572)	381 (172–573)	405.5 (196–594)	468 (242–655)
Haptoglobin (mg/dL)	95 (<5–305)	80 (<5–223)	63.5 (<5–192)	65 (<5–130)
Apolipoprotein A1 (mg/dL)	126 (45–198)	127 (63–191)	116 (46–172)	108 (62–171)
Hyaluronic acid (μg/L)	37.5 (<5–1260)	68 (5–1000)	140.5 (23–2610)	159 (33–364)
TIMP-1 (ng/mL)	157.5 (77–301)	172 (89–355)	188.5 (99–430)	192 (112–320)
TIMP-2 (ng/mL)	70 (21–294)	73 (21–207)	89 (27–280)	76 (36–120)
Procollagen III peptide (U/mL)	0.73 (0.52–8.30)	0.81 (0.53–1.60)	1.00 (0.63–1.90)	1.00 (0.68–1.60)
Type IV collagen 7S (ng/mL)	3.9 (1.2–12.0)	4.5 (2.3–9.9)	5.8 (2.8–16.0)	6.1 (4.6–10.0)

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

recognize the stepwise progression of viral hepatitis from F1 through F4. This dichotomy (mild or severe) of chronic hepatitis C 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 C.

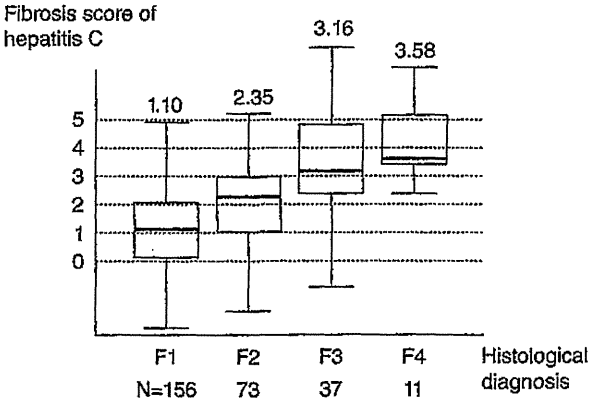

Figure 3 Box and whisker plots of fibrotic score of each group of histological fibrosis in the validation dataset. Fibrotic score of hepatitis C (FSC) was generated by the function, $z = 2.89 \times ln$ (type IV collagen 7S) (ng/mL) $- 0.011 \times$ (platelet count) ($\times10^3$/mm^3) $+ 0.79 \times ln$ (total bilirubin) (ng/mL) $+ 0.39 \times ln$ (hyaluronic acid) (μg/L) $- 1.87$.

This study was aimed to establish non-invasive evaluation and calculation of liver fibrosis for patients with chronic HCV infection. Although it was retrospectively performed as a multicenter study of eight institutions, judgment of histological diagnosis was independently performed by four pathologists in the other hospital, informed of nothing except for the patient's age, sex and positive HCV infection. Objective judgment of the histological staging and grading in sufficient biopsy specimens could be obtained.

As many as 581 patients with chronic hepatitis C 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 fibrotic markers: α2-macroglobulin, haptoglobin concentration, haptoglobin typing, apolipo-

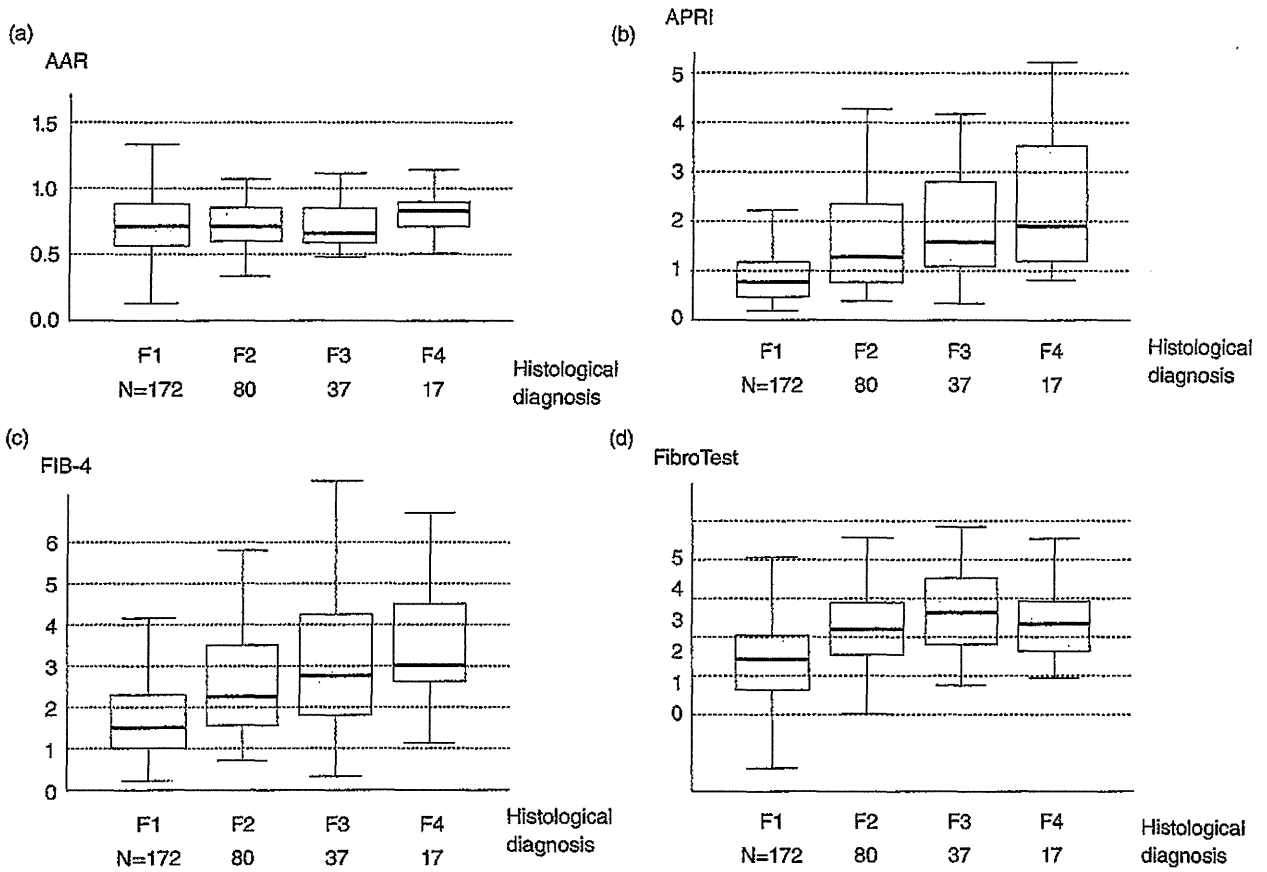

Figure 4 Previously published fibrotic scores: (a) aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ratio (AAR),[8] (b) AST-to-platelet ratio index (APRI), calculated by AST / (upper limit of normal of AST) / (platelet count [$\times 10^9$/L]) $\times$ 100.[12] (c) FIB-4 score, calculated by age $\times$ AST [IU/L] / (platelet count [$\times 10^9$/L] $\times$ ALT [IU/L]$^{0.5}$).[13] (d) FibroTest score regression coefficient was: $Z = 4.467 \times \log^{10}$ (α2-macroglobulin [g/L]) $- 1.357 \times \log^{10}$ (haptoglobin [g/L]) $+ 1.017 \times \log^{10}$ [γ-glutamyltransferase [GGT] [IU/L]) $+ 0.0281 \times$ (age [years]) $+ 1.737 \times \log^{10}$ (bilirubin [μm/L]) $- 1.184 \times \log^{10}$ (apolipoprotein A1 [g/L]) $+ 0.301 \times$ (sex [female = 0, male = 1]) $- 5.54$.[9]

protein 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, bilirubin and hyaluronic acid. A constant numeral (−1.87) was finally adjusted in the regression equation in order to obtain fitted figures for fibrotic stages of F1, F2, F3 and F4. From the magnitude of the standardized partial regression coefficient of individual variable in the function, *ln* (type IV collagen 7S) demonstrated the most potent contribution toward the prediction of liver fibrosis. Platelet count and *ln* (bilirubin) proved to be the second and third distinctive power in the model, respectively.

The obtained figure of FSC was generated to imitate actual "F factor" of histological staging. FSC was sufficiently fitted to actual fibrotic stages with certain overlapping as was usually found in histological ambiguity judged by pathologists. Because judgment of fibrosis in chronic hepatitis often shows a transitional histological staging, pathological examination could not always achieve a clear-cut diagnosis discriminating F1, F2, F3 or F4. Considering the limitation of pathological difficulty in differentiation of the four continuous disease entities, the obtained regression function showed satisfactory high accuracy rates in the prediction of liver disease severity. FSC can provide one or two decimal places (e.g. 2.4 or 2.46) and the utility of the score is possibly higher

than mere histological staging of F1, F2, F3 or F4. The reproducibility was confirmed by the remaining 276 patients' data obtained from the other seven hospitals. Although the validation data were collected from different geographic area and different chronologic situation, FSC showed similar results in prediction of histological staging.

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

The score can be calculated for any patients with chronic HCV 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 with a slight degree of chronic hepatitis with a tiny fibrotic change as F0. Very severe fibrosis may be calculated as more than 4.00, which is an imaginable and nonsense number in the scoring system of fibrosis. FSC is, however, very useful and valuable in real clinical setting. Estimation of severity of liver fibrosis in outpatient clinics, evaluation of natural progression of patients' fibrosis over 10 years, and assessment of a long-term administration of interferon in patients with chronic hepatitis C from the viewpoint of fibrotic change. In this study, because certain patients actually had a history of interferon administration, regression of liver fibrosis during and after the treatment could be assessed when prior sera were available for serial evaluation of FSC. We can also expect the usefulness of evaluation of carcinogenic risk after sustained virological response, and stage progression with alcohol intake or obesity-induced steatosis. Recent development of new directly acting antiviral agents require evaluation for long-term histological advantage, for aggravation of hepatitis stage during viral and biochemical breakthrough caused by HCV mutation, estimation of future carcinogenic risk, and even for the best way of management of patients with chronic hepatitis C. FSC seems one of the ideal methods of approximation for fibrotic stage of chronic hepatitis C. 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 HCV-related chronic liver disease, this equation would not be suitable for the recognition of HBV-related chronic liver disease,[22] alcoholic liver disease and other congenital or autoimmune liver diseases. To recognize the latter diseases, other studies about individual diseases must be performed.

We compared the usefulness of the FSC with that of other fibrotic scores.[8,9,12,13] More simple and inexpensive AAR or APRI could not well estimate fibrotic stages with poor correlation coefficients of 0.021 and 0.462, which were much lower than the coefficient of FSC of 0.572. FibroTest, which contained three costly fibrotic markers (α2-macroglobulin, haptoglobin and apolipoprotein A1), also showed a low correlation coefficient of 0.415, suggesting that the usefulness was limited in HCV positive Asian patients. Although FIB-4 demonstrated the best coefficient of 0.440 among the fibrotic scores, significant overlaps were found between neighboring stages and obtained scores were not coordinated for rea17l histological classification. Because this study also measured those special markers included in FibroTest, the ability of discrimination of fibrotic stages could be compared among the five fibrotic scoring systems.

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

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Seroclearance rate of hepatitis B surface antigen in 2,112 patients with chronic hepatitis in Japan during long-term follow-up

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Abstract
Background Rate of hepatitis B surface antigen (HBsAg) seroclearance was determined in 2,112 Japanese patients with chronic hepatitis B who were followed up for at least 15 years.
Methods Patients had a median age of 37 years and included 1,431 (67.8 %) men. Median values were AST/ALT, 43/62 IU/L; platelet counts, 182 × 10^3/mm^3; HBsAg, 3,400 IU/mL; and hepatitis B virus (HBV) DNA, 6.2 log copies/mL. Factors influencing HBsAg seroclearance were evaluated by the Cox proportional model and annual rate of HBsAg seroclearance by the Kaplan–Meier life table method.
Results The overall annual rate of HBsAg seroclearance was 1.75 % in 2,112 patients; it was 1.65 % in 1,130 untreated and 2.05 % in 982 treated patients ($p = 0.289$). In untreated patients, seroclearance was influenced by age, no HBV infections in third-degree or closer relatives, and HBsAg levels in univariate analysis. Seroclearance was influenced by a median age ≥50 years [relative risk (RR) 1.61 ($p = 0.018$)] and HBsAg ≤2,000 IU/mL [RR 1.77 ($p = 0.014$)] in multivariate analysis. In treated patients, age, male gender, no HBV infections in third-degree or closer relatives, interferon therapy, chronic hepatitis, high AST and γ-GTP levels, low platelet counts, hepatitis B e antigen (HBeAg)-negative status, low HBsAg levels and the wild-type precore sequence significantly influenced HBsAg seroclearance. In multivariate analysis, no family history [RR 2.22 ($p = 0.006$)], interferon treatment [RR 3.15 ($p < 0.001$)], and HBeAg-negative status [RR 3.75 ($p < 0.001$)] significantly influenced HBsAg seroclearance.
Conclusions In this retrospective cohort study, the annual rate of HBsAg seroclearance was 1.65 % in untreated patients and 2.05 % in treated patients.

Keywords Seroclearance · Hepatitis B surface antigen · Hepatitis B virus · Chronic hepatitis B

Abbreviations

ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ETV	Entecavir
HBeAg	Hepatitis B e antigen
HBcrAg	Hepatitis B core-related antigen
HBV	Hepatitis B virus
HBV DNA	Hepatitis B virus DNA
HBsAg	Hepatitis B surface antigen
IFN	Interferon
LAM	Lamivudine

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Introduction

Worldwide, an estimated 400 million people are infected with hepatitis B virus (HBV) persistently. HBV infection is a common disease that can induce a chronic carrier state

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and is associated with the risk of developing progressive disease and hepatocellular carcinoma (HCC) [1–5]. In regions highly endemic for HBV, such as Asia and Africa, the persistent carrier state is established by perinatal transmission or early in infancy. Carriers serve as the reservoir of HBV in the community and can spread the infection to susceptible individuals. The incidence of HCC is decreased extremely by eradicating HBV from the circulation that is responsible for liver damage [6–9]. In Japan, interferon (IFN) was introduced for the treatment of persistent HBV infections, and long-term IFN increased seroclearance of hepatitis B surface antigen (HBsAg) [10]. Since 2000, the effect of long-term nucleot(s)ide analogues, such as lamivudine [11, 12] and entecavir [13], on HBsAg seroclearance has been monitored in Japan.

In the current study, we followed untreated or treated patients for at least 15 years. We evaluated the seroclearance of HBsAg, achieved in both groups of patients, by using highly sensitive assays. Our aim was to determine factors that can lead to HBsAg seroclearance and to elucidate the factors associated with its success.

Patients and methods

Patients

During at least 15 years from 1968, 2,112 consecutive patients, chronically mono-infected with HBV (confirmed by HBsAg-positivity for at least 6 months) were followed at the Department of Hepatology, Toranomon Hospital, in Metropolitan Tokyo. Patients met the following inclusion and exclusion criteria: (1) negativity for hepatitis C antibody and/or hepatitis C virus RNA by polymerase chain reaction (PCR) in the serum; (2) no history of HCC; and (3) no history of autoimmune hepatitis, alcohol liver disease, hemochromatosis, or chronic liver disease other than chronic hepatitis B. Thus, the 2,112 patients were enrolled in this cohort study. A written informed consent was obtained from each patient. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved a priori by the institution's human research committee.

Treatment

Nine hundred and eighty-two patients received antiviral treatments. Of them, 156 patients received prednisolone (PSL) 40 mg daily for 1 week, 30 mg daily for 1 week, 20 mg daily for 1 week, and then 10 mg daily for 1 week until it was abruptly withdrawn (total 700 mg). A total of 428 patients received 100 mg lamivudine (LAM) daily as an initial therapy. In total, 333 patients received 3–12 MU of IFN-α or IFN-β. The durations and regimens of treatment were as follows: daily for 2 or 4 weeks and then 2 or 3 times per week for 26–104 weeks. The median duration of treatment was 26 weeks (range 4–981). There were 190 (57 %) patients who received multiple treatments of IFN.

LAM treatment was continued as a rule; median duration of LAM treatment was 75 months (55–102). LAM-resistant rtM204I/V mutants developed in 151 (35 %) of the 428 patients, and they were provided with adefovir dipivoxil (10 mg) added on LAM, as a rescue therapy. The remaining patients continued to receive LAM monotherapy. In addition, 65 patients received 0.5 mg entecavir (ETV) daily as an initial therapy. ETV treatment was continued as a rule, and median duration of ETV treatment was 45 months (1.0–104).

Markers of HBV infection

Serum HBsAg titers were determined annually using ARCHITECT HBsAg QT assay kits (Abbott Laboratories, Tokyo, Japan), which have a lower limit of detection of 0.05 IU/mL and an upper limit of detection of 250 IU/mL. To expand the upper limit from 250 to 125,000 IU/mL, serum samples going off the scale were diluted stepwise to 1:20 and 1:500 with ARCHITECT diluents following instructions from the manufacturer.

Hepatitis B e antigen (HBeAg) was determined by enzyme-linked immunosorbent assay with a commercial kit (HBeAg EIA; Institute of Immunology, Tokyo, Japan). HBV DNA was quantified using the Amplicor monitor assay (Roche Diagnostics, Tokyo, Japan) with a dynamic range of 2.6–7.6 log copies/mL, or COBAS TaqMan HBV v.2.0 (Roche Diagnostics, Tokyo, Japan) with a dynamic range of 2.1–9.0 log copies/mL. Hepatitis B core-related antigen (HBcrAg) was determined by chemiluminescence enzyme immunoassay (CLEIA) with the HBcrAg assay kit (Fujirebio Inc., Tokyo, Japan). A commercial kit (HBV Genotype EIA; Institute of Immunology, Tokyo, Japan) was used to serologically determine HBV genotypes by the combination of epitopes expressed on the pre-S2 region product, which is specific for each of the 7 major genotypes (A–G).

Statistical analysis

Baseline data were obtained on the day of the first visit in untreated patients. In patients who received antivirals, baseline data were obtained at the start of the first day of treatment. Categorical data were compared between groups by chi-squared or Fisher's exact tests. Continuous variables with a nonparametric distribution were analyzed by Mann–Whitney U tests, whereas those with a parametric distribution were analyzed by the Student's t test. Cox

regression analyses were used to assess variables that were significantly associated with HBsAg seroclearance. All baseline factors that were found to be significantly associated with HBsAg seroclearance by univariate analysis were entered into a multivariate analysis. Independent baseline factors associated with the seroclearance of HBsAg were evaluated using a stepwise Cox regression analysis. We then performed a time-dependent Cox regression to analyze independent factors associated with HBsAg seroclearance while on-treatment factors and independent baseline factors had been adjusted.

Cumulative HBsAg seroclearance rates were analyzed using the Kaplan–Meier method; differences in the resulting curves were evaluated using log-rank tests. Significance was defined as $p < 0.05$ for all two-tailed tests. Data analysis was performed with the SPSS software package version 11.0.1 J (SPSS Inc., Chicago, IL, USA).

Results

Baseline characteristics in the 2,112 patients

The baseline characteristics of studied patients are shown in Table 1. They had a median age of 37 years (range 1–81), included 1,431 (67.8 %) men, and 2,031 (96.2 %) of them had chronic hepatitis. Their baseline values were AST/ALT, 43 (3–2,192)/62 (2–3,020 IU/L); γ-GTP, 27 (4–1,494) IU/L; platelet counts, 182 (40–483) × 10^3/mm^3; and HBV markers were HBsAg, 3,400 (0.06–27,700) IU/mL; and HBV DNA, 6.2 (<2.1 to >9.1) log copies/mL. HBeAg was not detectable in 5.4 % of studied patients, and the distribution of genotypes A/B/C/others was 4.5:15.6:79.6:0.3 %.

The HBsAg seroclearance rate analyzed by the Kaplan–Meier method was 9 % in 5 years, 17 % in 10 years, 27 % in 15 years, 35 % in 20 years, 44 % in 25 years, and 54 % in 30 years. The annual rate of HBsAg seroclearance was 1.75 % during 20 years (Fig. 1).

In the 2,112 patients, factors influencing HBsAg seroclearance in univariate analysis by the Cox regression analyses were cirrhosis [relative risk (RR) 2.40 ($p = 0.014$)]; HBeAg negative [RR 3.01 ($p = 0.001$)]; and HBsAg ≤2,000 IU/mL [RR 2.13 ($p = 0.004$)]. In multivariate analyses, only 2 factors contributed to HBsAg seroclearance: HBeAg negative [RR 1.81 ($p < 0.001$)]; and HBsAg ≤2,000 IU/mL [RR 2.60 ($p < 0.001$)] (Table 2).

Untreated patients and treated patients

Differences in the baseline characteristics between 1,130 untreated and 982 treated patients are shown in Table 3: age [31 years vs. 36 ($p < 0.001$)]; male gender [62.4 vs. 71.9 % ($p < 0.001$)]; AST [median 27 vs. 56 IU/L ($p < 0.001$)]; ALT [median 28 vs. 96 IU/L ($p < 0.001$)]; γ-GTP [median 20 vs. 45 IU/L ($p < 0.001$)]; total bilirubin

Table 1 Baseline characteristics 2,112 patients infected with HBV followed for longer than 15 years

Features at the baseline	Patients ($n = 2,112$)
Demographic data	
Age (years)	37 (1–81)
Men	1,431 (67.8 %)
Liver disease	
Chronic hepatitis	2,031 (96.2 %)
Cirrhosis	81 (3.8 %)
Laboratory data	
AST (IU/L)	43 (3–2,192)
ALT (IU/L)	62 (2–3,020)
γ-GTP (IU/L)	27 (4–1,494)
Total bilirubin (mg/dL)	0.7 (0.1–21.2)
Albumin (g/dL)	4.3 (1.1–5.8)
Platelets (×10^3/mm^3)	182 (40–483)
α-Fetoprotein (μg/L)	4 (1–2,060)
HBV markers	
HBeAg-negative status	1,169 (55.4 %)
HBsAg (IU/mL)	3,400 (0.06–277,000)
HBcrAg (log U/mL)	5.4 (<3.0 to >6.8)
Genotypes (A/B/C/others)	4.5 %/15.6 %/79.6 %/0.3 %
HBV DNA (log copies/mL)	6.2 (<2.1 to >9.1)

Median values with the range in parentheses or numbers with the percentage in parentheses are given

HBV hepatitis B virus, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *γ-GTP* γ-guanosine triphosphate, *HBeAg* hepatitis B e antigen, *HBsAg* hepatitis B surface antigen, *HBcrAg* hepatitis B core-related antigen

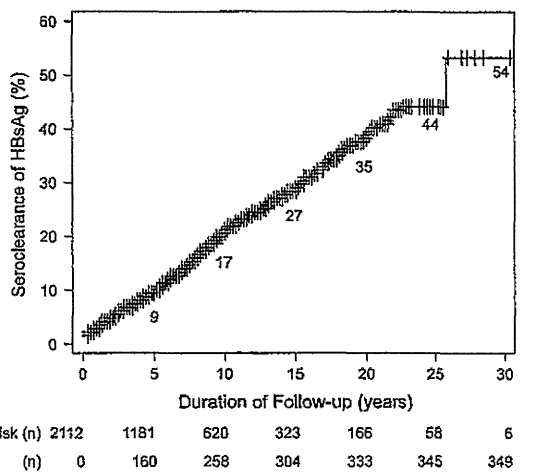


Fig. 1 Seroclearance of HBsAg in the 2,112 patients studied. Numbers of patients at risk and those of patients who lost HBsAg are indicated below each time point

Table 2 Factors influencing the seroclearance of HBsAg in 2,112 patients evaluated by time-dependent uni- and multivariate analyses

Factors	Univariate analysis HBsAg clearance Relative risk (95 % CI)	*p* value	Multivariate analysis HBsAg clearance Relative risk (95 % CI)	*p* value
Age ≥50 years	1.06 (0.64–1.76)	0.824		
Male gender	1.15 (0.69–1.90)	0.594		
No HBV infection in family	1.55 (0.93–2.57)	0.092		
Treatment	1.26 (0.72–2.19)	0.413		
Cirrhosis	2.40 (1.20–4.83)	0.014		
AST ≥50 IU/L	1.30 (0.66–2.57)	0.454		
ALT ≥50 IU/L	1.81 (0.89–3.70)	0.104		
γ-GTP ≥20 IU/L	1.26 (0.72–2.23)	0.418		
Total bilirubin ≥1 mg/dL	1.39 (0.69–2.79)	0.358		
Albumin ≥4 g/dL	1.03 (0.58–1.81)	0.927		
Platelets >150 × 10^3/mm^3	1.22 (0.68–2.18)	0.501		
α-Fetoprotein ≤10 μg/L	1.06 (0.59–1.89)	0.845		
Genotype A or B, C	1.55 (0.86–2.76)	0.142		
HBeAg-negative status	3.01 (0.79–2.07)	0.001	1.81 (1.30–2.77)	<0.001
HBV DNA ≥5 log copies/mL	1.17 (0.64–2.15)	0.612		
HBsAg ≤2,000 IU/mL	2.13 (1.27–3.56)	0.004	2.60 (1.94–3.50)	<0.001
HBcrAg ≥4 log U/mL	1.11 (0.61–2.03)	0.731		
Wild-type precore sequence	0.98 (0.59–1.53)	0.964		
Wild-type core promoter sequence	2.74 (0.80–9.30)	0.104		

Wild-type precore sequence, G1896; wild-type core promoter sequence, A1762/G1764

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *γ-GTP* γ-guanosine triphosphate, *HBeAg* hepatitis B e antigen, *HBsAg* hepatitis B surface antigen, *HBcrAg* hepatitis B core-related antigen

[median 0.5 vs. 0.7 mg/dL ($p < 0.001$)]; albumin [median 4.4 vs. 4.3 g/dL ($p < 0.001$)]; platelets [median 202 vs. 181 × 10^3/mm^3 ($p < 0.001$)]; α-fetoprotein [median 4 vs. 4 μg/L ($p < 0.001$)]; HBeAg-negative status [75.8 vs. 31.8 % ($p < 0.001$)]; HBsAg levels [median 2,240 vs. 5,270 IU/mL ($p < 0.001$)]; HBcrAg [median 3.6 vs. >6.8 log U/mL ($p < 0.001$)]; distribution of genotypes A/B/C/others (5.7/20.0/72.6/1.7 vs. 3.4/11.1/84.9/0.5 %, $p < 0.001$); and HBV DNA [median 4.7 vs. 8.0 log copies/mL ($p < 0.001$)].

The rate of HBsAg seroclearance in treated patients was 8 % in 5 years, 20 % in 10 years, 28 % in 15 years, 41 % in 20 years, 49 % in 25 years, and 49 % in 30 years, with an annual HBsAg seroclearance rate of 2.05 % (Fig. 2). The rate in untreated patients was 9 % in 5 years, 18 % in 10 years, 26 % in 15 years, 33 % in 20 years, 42 % in 25 years, and 56 % in 30 years, with an annual HBsAg seroclearance rate of 1.65 %. No differences in the annual HBsAg seroclearance rate were noted between treated and untreated patients ($p = 0.289$).

HBsAg seroclearance in untreated patients

In the 1,130 untreated patients, HBsAg persisted in 930 (82.3 %), whereas HBsAg seroclearance occurred in 200 (17.7 %). In the baseline characteristics, significant differences were found for age ($p < 0.001$), male gender ($p = 0.003$), chronic hepatitis ($p = 0.020$), γ-GTP ($p < 0.001$), albumin ($p = 0.004$), HBV genotypes ($p < 0.001$), HBeAg-negative status ($p < 0.001$), HBV DNA ($p < 0.001$), HBsAg level ($p < 0.001$), HBcrAg ($p < 0.001$), precore wild-type ($p < 0.001$), and core promoter wild-type ($p = 0.001$) (Table 4).

Factors contributing to HBsAg seroclearance in untreated patients

In the 1,130 untreated patients, factors influencing HBsAg seroclearance in univariate analysis by the Cox regression analyses were age ≥50 [RR 1.63 ($p = 0.002$)]; no family history in third-degree or closer relatives [RR 1.38 ($p = 0.037$)]; and HBsAg ≤2,000 IU/mL [RR 1.87 ($p < 0.006$)].

In multivariate analyses, only 2 factors contributed to HBsAg seroclearance: age ≥50 [RR 1.61 ($p = 0.018$)] and HBsAg ≤2,000 IU/mL [RR 1.77 ($p = 0.014$)] (Table 5).

HBsAg seroclearance in treated patients

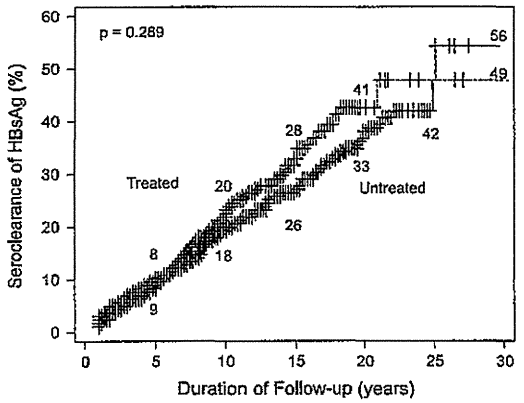
In the 982 treated patients, HBsAg persisted in 833 (84.8 %). HBsAg seroclearance occurred in 149 (15.2 %). In the baseline characteristics, significant difference were found for male gender ($p = 0.004$), no family history in third-degree or closer relatives ($p = 0.010$), chronic hepatitis ($p = 0.001$), AST ($p = 0.010$), γ-GTP ($p = 0.023$), platelet counts ($p < 0.001$), HBeAg-negative status

Table 3 Baseline characteristics in untreated and treated patients

Features at the baseline	Untreated (n = 1,130)	Treated (n = 982)	Differences p value
Age (years)	31 (1–81)	36 (6–75)	<0.001
Men	705 (62.4 %)	726 (71.9 %)	<0.001
Chronic hepatitis	1,094 (96.8 %)	937 (96.4 %)	0.079
Cirrhosis	36 (3.2 %)	45 (3.6 %)	
AST (IU/L)	27 (3–1,776)	56 (6–2,192)	<0.001
ALT (IU/L)	28 (2–3,020)	96 (8–2,740)	<0.001
γ-GTP (IU/L)	20 (4–1,494)	45 (4–1,278)	<0.001
Total bilirubin (mg/dL)	0.5 (0.1–20.1)	0.7 (0.2–21.2)	<0.001
Albumin (g/dL)	4.4 (2.2–5.8)	4.3 (1.1–5.4)	<0.001
Platelets ($\times 10^3$/mm^3)	202 (40–443)	181 (40–483)	<0.001
α-Fetoprotein (μg/L)	4 (1–2,060)	4 (1–1,610)	<0.001
HBeAg-negative status	857 (75.8 %)	312 (31.8 %)	<0.001
HBsAg (IU/mL)	2,240 (0.06–141,000)	5,270 (0.09–277,000)	<0.001
HBcrAg (log U/mL)	3.6 (<3.0 to >6.8)	> 6.8 (<3.0 to >6.8)	<0.001
Genotypes [A/B/C/others (%)]	5.7/20.0/72.6/1.7	3.4/11.1/84.9/0.5	<0.001
HBV DNA (log copies/mL)	4.7 (<2.1 to >9.1)	8.0 (<2.1 to >9.1)	<0.001

Median values with the range in parentheses or numbers with the percentage in parentheses are given

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *γ-GTP* γ-guanosine triphosphate, *HBeAg* hepatitis B e antigen, *HBsAg* hepatitis B surface antigen, *HBcrAg* hepatitis B core-related antigen



Treated							
Patients at Risk (n)	982	529	221	104	39	8	3
HBsAg Lost (n)	0	66	114	133	145	148	149
Untreated							
Patients at Risk (n)	1130	652	399	219	127	50	3
HBsAg Lost (n)	0	91	142	170	187	197	200

Fig. 2 Comparison of HBsAg seroclearance rates between 982 treated and 1,130 untreated patients. Numbers of patients at risk and those of patients who lost HBsAg are indicated below each time point

($p < 0.001$), HBV DNA ($p = 0.002$), HBsAg ($p < 0.001$), HBcrAg ($p = 0.003$), and precore wild-type ($p = 0.013$) (Table 6).

Factors contributing to HBsAg seroclearance in treated patients

In the 982 treated patients, factors influencing HBsAg seroclearance in univariate analysis by the Cox regression analyses were age ≥50 [RR 1.91 ($p = 0.001$)]; male gender [RR 2.14 ($p = 0.001$)], no family history in third-degree or closer relatives [RR 1.58 ($p = 0.005$)]; previous treatment with interferon [RR 2.13 ($p < 0.001$)]; chronic hepatitis [RR 3.12 ($p < 0.001$)]; AST ≥50 IU/L [RR 1.47 ($p = 0.031$)]; γ-GTP ≥20 IU/L [RR 1.87 ($p = 0.001$)]; platelets ≤150 × 10^3/mm^3 [RR 2.10 ($p < 0.001$)]; HBeAg-negative status [RR 2.53 ($p < 0.00$)]; HBV DNA ≤5 log copies/mL [RR 2.07 ($p = 0.001$)]; HBsAg ≤2,000 IU/mL [RR 2.29 ($p < 0.001$)]; HBcrAg ≤4 log U/mL [RR 2.28 ($p = 0.003$)]; and the wild-type precore sequence [RR 2.04 ($p = 0.011$)].

In multivariate analysis, only 3 factors contributed to HBsAg seroclearance: no family history in third-degree or closer relatives [RR 2.22 ($p = 0.006$)]; previous treatments with interferon [RR 3.15 ($p < 0.001$)]; and HBeAg-negative status [RR 3.75 ($p < 0.001$)] (Table 7).

Discussion

In Japan, perinatal materno-fetal transmission was the main route of HBV infection, but this transmission has been prevented since 1986 by the national campaign to prevent it by immunoprophylaxis with combined passive-active immunization of babies born to HBeAg-positive carrier mothers. However, HCC develops in about 10 % of the patients who have established chronic HBV infection by materno-fetal infection or through child-to-child transmission. Hence, HBsAg seroclearance is crucially required for preventing the development of cirrhosis followed by HCC.

In the present study, we analyzed 2,112 patients with persistent HBV infection to establish the factors

Table 4 Differences between the baseline characteristics of 917 untreated patients in whom HBsAg persisted and 213 those who lost HBsAg

Features at the baseline	HBsAg persisted ($n = 917$)	HBsAg lost ($n = 213$)	Differences p value
Age (years)	37 (1–81)	44 (0–80)	<0.001
Men	553 (60.3 %)	152 (71.4 %)	0.003
HBV in family members	349 (38.1 %)	76 (35.7 %)	0.509
Chronic hepatitis	893 (97.4 %)	201 (94.4 %)	0.020
AST (IU/L)	27 (3–1,144)	25 (6–1,776)	0.283
ALT (IU/L)	28 (6–1,960)	27 (6–3,020)	0.389
γ-GTP (IU/L)	22 (1–1,494)	29 (4–1,092)	<0.001
Total bilirubin (mg/dL)	0.6 (0.2–20.1)	0.7 (0.1–4.0)	0.257
Albumin (g/dL)	4.3 (2.0–5.3)	4.4 (1.6–5.7)	0.004
Platelets ($\times 10^3$/mm^3)	203 (40–443)	203 (33–417)	0.473
α-Fetoprotein (μg/L)	3 (1–2,060)	1 (1–478)	0.373
Genotypes [A/B/C/others (%)]	5.7/19.0/73.3/1.9	5.5/24.7/69.2/0.7	<0.001
HBeAg-negative status	663 (72.3 %)	194 (91.1 %)	<0.001
HBV DNA (log copies/mL)	4.9 (<2.1 to >9.1)	3.8 (<2.1 to >9.1)	<0.001
HBsAg (IU/mL)	3,100 (1.94–141,000)	149 (0.06–88,800)	<0.001
HBcrAg (log U/mL)	3.9 (<3.0 to >6.8)	2.9 (<3.0 to >6.8)	<0.001
Wild-type precore sequence	441 (48.1 %)	160 (75.0 %)	<0.001
Wild-type core promoter sequence	320 (34.9 %)	47 (22.0 %)	0.001

Wild-type precore sequence, G1896; wild-type core promoter sequence, A1762/G1764

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *γ-GTP* γ-guanosine triphosphate, *HBeAg* hepatitis B e antigen, *HBsAg* hepatitis B surface antigen, *HBcrAg* hepatitis B core-related antigen

Table 5 Factors influencing the seroclearance of HBsAg in untreated patients evaluated by time-dependent uni- and multivariate analyses

Factors	Univariate analysis HBsAg clearance Relative risk (95 % CI)	p value	Multivariate analysis HBsAg clearance Relative risk (95 % CI)	p value
Age ≥50 years	1.63 (1.19–2.23)	0.002	1.61 (1.09–2.37)	0.018
Male gender	1.08 (0.79–1.48)	0.618		
No HBV infection in family	1.38 (1.02–1.86)	0.037		
Cirrhosis	1.19 (0.73–1.93)	0.484		
AST ≥50 IU/L	1.01 (0.70–1.45)	0.979		
ALT ≥50 IU/L	0.93 (0.68–1.27)	0.633		
γ-GTP ≥20 IU/L	1.17 (0.85–1.61)	0.330		
Total bilirubin ≥1 mg/dL	1.41 (0.80–2.49)	0.239		
Albumin ≥4 g/dL	0.78 (0.51–1.18)	0.239		
Platelets >150 × 10^3/mm^3	0.99 (0.67–1.46)	0.946		
α-Fetoprotein ≤10 μg/L	0.84 (0.48–1.47)	0.543		
Genotype A or B	1.17 (0.81–1.69)	0.410		
HBeAg-negative status	0.78 (0.79–2.07)	0.314		
HBV DNA ≥5 log copies/mL	0.84 (0.58–1.24)	0.383		
HBsAg ≤2,000 IU/mL	1.87 (1.19–2.91)	0.006	1.77 (1.12–2.77)	0.014
HBcrAg ≥4 log U/mL	0.85 (0.50–1.45)	0.555		
Wild-type precore sequence	0.99 (0.60–1.52)	0.967		
Wild-type core promoter sequence	0.78 (0.35–1.73)	0.538		

Wild-type precore sequence, G1896; wild-type core promoter sequence, A1762/G1764

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *γ-GTP* γ-guanosine triphosphate, *HBeAg* hepatitis B e antigen, *HBsAg* hepatitis B surface antigen, *HBcrAg* hepatitis B core-related antigen

contributing to HBsAg seroclearance. The overall rate of HBsAg seroclearance was 1.75 % annually. The annual seroclearance rates of HBsAg are reported to be 1.7 % in Korea [14] and 1.6 % in Taiwan [15–17], as well as 2.5 % in Goto Islands of Japan, where HBV infections are very prevalent [18]. In 1,271 natives in Alaska, the rate of HBsAg seroclearance was 0.7 % annually [19]. These differences could be ascribed, in part, to HBV genotypes distinct among Asian countries and Alaska. Since treatment with IFN and/or nucleot(s)ide analogues has suppressive effects on the development of HCC [6, 20], they may influence HBsAg seroclearance.

Table 6 Differences in baseline characteristics between the 833 treated patients in whom HBsAg persisted and 149 those who lost HBsAg

Features at the baseline	HBsAg persisted ($n = 833$)	HBsAg lost ($n = 149$)	Differences p value
Age (years)	41 (13–88)	43 (17–71)	0.285
Men	601 (72.2 %)	124 (83.2 %)	0.004
HBV in family members	496 (59.6 %)	72 (48.3 %)	0.010
Chronic hepatitis	802 (96.3 %)	134 (89.9 %)	0.001
AST (IU/L)	54 (6–2,192)	78 (7–888)	0.010
ALT (IU/L)	93 (8–2,740)	118 (8–1,700)	0.117
γ-GTP (IU/L)	44 (4–1,278)	46 (4–1,278)	0.023
Total bilirubin (mg/dL)	0.7 (0.2–21.2)	0.7 (0.3–8.4)	0.273
Albumin (g/dL)	4.3 (1.1–5.4)	4.5 (1.4–5.3)	0.281
Platelets ($\times 10^3$/mm^3)	182 (40–483)	171 (50–391)	<0.001
α-Fetoprotein (μg/L)	4 (1–1,610)	4 (1–765)	0.682
Genotypes [A/B/C/others (%)]	3.2/10.7/85.1/1.0	5.1/12.4/81.6/0.9	0.565
HBeAg-negative status	230 (27.6 %)	79 (53.0 %)	<0.001
HBV DNA (log copies/mL)	7.8 (<2.1 to >9.1)	8.3 (<2.1 to >9.1)	0.002
HBsAg (IU/mL)	7,880 (0.04–277,000)	1,380 (0.04–188,000)	<0.001
HBcrAg (log U/mL)	6.9 (<3.0 to >6.8)	5.9 (<3.0 to >6.8)	0.003
Wild-type precore sequence	554 (66.6 %)	61 (41.2 %)	0.013
Wild-type core promoter sequence	274 (32.9 %)	67 (45.0 %)	0.836

Wild-type precore sequence, G1896; wild-type core promoter sequence, A1762/G1764

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *γ-GTP* γ-guanosine triphosphate, *HBeAg* hepatitis B e antigen, *HBsAg* hepatitis B surface antigen, *HBcrAg* hepatitis B core-related antigen

Table 7 Factors influencing the seroclearance of HBsAg in treated patients evaluated by time-dependent uni- and multivariate analyses

Factors	Univariate analysis HBsAg clearance Relative risk (95 % CI)	p value	Multivariate analysis HBsAg clearance Relative risk (95 % CI)	p value
Age ≥50 years	1.91 (1.32–2.77)	0.001		
Male gender	2.14 (1.37–3.33)	0.001		
No HBV infection in family	1.58 (1.15–2.19)	0.005	2.22 (2.32–3.94)	0.006
Treatments (interferon vs. others)	2.13 (1.53–2.98)	<0.001	3.15 (1.69–5.87)	<0.001
Chronic hepatitis	3.12 (2.05–4.74)	<0.001		
AST ≥50 IU/L	1.47 (1.04–2.09)	0.031		
ALT ≥50 IU/L	1.29 (0.82–1.92)	0.201		
γ-GTP ≥20 IU/L	1.87 (1.30–2.70)	0.001		
Total bilirubin ≥1 mg/dL	1.35 (0.87–2.08)	0.179		
Albumin ≥4 g/dL	1.11 (0.66–1.86)	0.688		
Platelets ≤150 × 10^3/mm^3	2.10 (1.49–2.96)	<0.001		
α-Fetoprotein ≤10 μg/L	1.33 (0.92–1.92)	0.136		
Genotype A or B vs. others	1.16 (0.74–1.82)	0.529		
HBeAg-negative status	2.53 (1.83–3.50)	<0.001	3.75 (2.09–6.74)	<0.001
HBV DNA ≤5 log copies/mL	2.07 (1.37–3.13)	0.001		
HBsAg ≤2,000 IU/mL	2.29 (1.52–3.47)	<0.001		
HBcrAg ≤4 log U/mL	2.28 (1.31–3.97)	0.003		
Wild-type precore sequence	2.04 (1.18–3.55)	0.011		
Wild-type core promoter sequence	1.18 (0.63–2.21)	0.608		

Wild-type precore sequence, G1896; wild-type core promoter sequence, A176.2/G1764

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *γ-GTP* γ-guanosine triphosphate, *HBeAg* hepatitis B e antigen, *HBsAg* hepatitis B surface antigen, *HBcrAg* hepatitis B core-related antigen

Therefore, we went on to extend our analysis to untreated patients and those treated with IFN or nucleotide analogues separately. Criteria for upper or lower levels of each parameter were set, taking into consideration the median value or a cutoff value with the lowest p value of the entire 2,112-patient cohort (Table 1), and unified for untreated and treated patients (Tables 5, 7).

Firstly, in the univariate analysis, age, no family history of HBV infection in third-degree or closer relatives, and decreased HBsAg levels lowered the annual rate of HBsAg seroclearance significantly. In multivariate analysis, age $\geq$50 years (RR 1.61, $p = 0.018$) and HBsAg $\leq$2,000 IU/mL (RR 1.77, $p = 0.014$) decreased the annual rate of HBsAg seroclearance significantly. Kato et al. [18] reported high HBsAg seroclearance rates in patients over 40 or over 50 years; in our patients, also, age $\geq$50 years increased RR to 1.61 ($p = 0.018$). As for HBsAg and HBV DNA, low HBsAg and HBV DNA levels increased the HBsAg seroclearance rate to 37.7 %, and therefore, low HBsAg levels are an important factor. In actuality, HBsAg levels $\leq$2,000 IU/mL increased the rate of HBsAg seroclearance with RR 1.77 ($p = 0.014$).

In treated patients, by contrast, age, the male gender, no HBV infections in third-degree or closer relatives, treatment with IFN, chronic hepatitis, high AST levels, high γ-GTP levels, low platelet counts, HBeAg-negative status, low HBsAg levels, low HBcrAg levels and the wild-type precore sequence were significant factors in univariate analysis. In multivariate analysis, no HBV infections in third-degree or closer relatives (RR 2.22, $p = 0.006$), interferon treatments (RR 3.15, $p < 0.001$), and HBeAg-negative status (RR 3.75, $p < 0.001$) were significant factors.

Thus, there were differences in factors predictive of the HBsAg loss between untreated and treated patients. Remarkably, age and HBsAg titer were independent factors in untreated patients, whereas family history and negative HBeAg were independent factors in treated patients. Since this work studied patients who were followed for a long time (>15 years), age and HBsAg titer were factors for clearance of HBsAg in untreated patients. Treated patients, in contrast, would have included more patients with HBeAg, with a good response to antiviral treatment, as well as those without family history who would have been infected with HBV with a sorter duration than those with family history. In other words, most untreated patients were those with favorable clinical course, in whom HBsAg titer gradually decreased and eventually lost it with time. In fact, there would be many such patients, the majority of whom do not visit hospitals and are unaware of HBV infection, who may have unapparent liver disease. Treated patients, on the other hand, would have had higher risks for cirrhosis and HCC, owing to elevated ALT/AST levels; this risk is especially high for patients with a family history of HBV [21]. Therefore, patients with family history would not be able to easily lose HBsAg.

In treated patients, IFN led to HBsAg loss more effectively than other treatments [RR 2.13, $p < 0.001$ (Table 7)]. The immunomodulatory activity of IFN, which is not shared by nucleot(s)ide analogues, would have accelerated the immune response to HBV required for the seroclearance of HBsAg. Of the 333 patients who received IFN, 190 (57 %) were treated with IFN multiply. In them, seroclearance of HBsAg was achieved in 49 of the 190 (26 %) patients with multiple IFN treatments in comparison with 41 of the 143 (29 %) with single IFN treatment. Owing to indications for IFN, patients who received IFN tended to be younger, without previous treatments and higher HBV DNA as well as ALT levels. They might have increased the rate of HBsAg loss that was higher with IFN than other treatments.

Since this is a retrospective cohort study of patients visiting our hospital for more than 15 years, and there has been so much innovation in the treatment of chronic hepatitis B during that period, treated and untreated patients have different backgrounds at the baseline. Hence, treated patients had higher ALT and HBV DNA levels with severer liver disease than untreated patients (Table 3). This might have been responsible, at least in part, for the failure in finding differences in the rate of HBsAg loss between untreated and treated patients (Fig. 2). Future studies will be aimed at analyzing contributing factors in treated and matched controls. This will allow us to analyze factors contributing to HBsAg seroclearance in the treatment of patients with chronic hepatitis B.

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Conflict of interest These authors disclose the following: Dr. Kumada reports having received investigator, lecture, and consulting fees from Dainippon Sumitomo Pharma Co., MSD KK, Bristol-Myers Squibb, Pharma International, Dentsu Sudler, and Hennessey Inc. Dr. Ikeda reports having received investigator, lecture, and consulting fees from Dainippon Sumitomo Pharma Co. No other potential conflicts of interest relevant to this article were reported.

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CASE REPORT

Gut and Liver, Vol. 7, No. 2, March 2013, pp. 246-251

Transcatheter Arterial Chemotherapy with Miriplatin for Hepatocellular Carcinoma Patients with Chronic Renal Failure: Report of Three Cases

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Miriplatin is a novel lipophilic platinum complex that was developed to treat hepatocellular carcinoma (HCC). Although HCC patients frequently have coexisting chronic renal failure, little prospective data are available regarding the clinical toxicity of chemotherapeutic agents used to treat HCC patients with chronic renal failure. In a phase II study, the plasma concentration of total platinum in patients who received miriplatin was very low, and no severe renal toxicity caused by miriplatin injection was reported. Here, we present three cases of HCC with stage 4 chronic renal failure who received transcatheter arterial chemotherapy with miriplatin. All cases were male, ages 72, 84, and 83 years, and had serum creatinine levels of 2.3, 1.6, and 1.9 mg/dL, respectively. Their estimated glomerular filtration rates were 21.9, 20.3, and 22.2 mL/min, respectively. All cases were treated for unresectable HCC with transcatheter arterial chemotherapy with miriplatin. No serious adverse events were observed, and serum creatinine levels did not elevate, even in the patient who experienced renal failure caused by cisplatin administration. These results might suggest that transcatheter arterial chemotherapy with miriplatin can be safely used in HCC patients with chronic renal failure. **(Gut Liver 2013;7:246-251)**

Key Words: Miriplatin; Chronic renal failure; Hepatocellular carcinoma

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common malignant diseases worldwide.[1] Since curative therapies, including resection, liver transplantation, and percutaneous ablation (percutaneous ethanol injection and radiofrequency ablation [RFA]) are applicable in only 30% to 40% of HCC patients, transcatheter arterial chemoembolization (TACE) has been recognized as an effective palliative treatment option for patients with advanced HCC.[2-7] HCC patients frequently have coexisting cirrhosis, which is a predisposing factor for the development of renal dysfunction due to intravascular volume depletion, inadequate renal vasoconstriction, and hyperaldosteronism.[8-13]

Little prospective data are available regarding the clinical toxicity of chemotherapeutic agents used to treat HCC patients with chronic renal failure. Although cisplatin is an effective anticancer drug that is widely used for the treatment of many malignancies, including HCC, it is associated with significant nephrotoxicity, particularly in patients with chronic renal failure.[2,7] Miriplatin is a novel cisplatin derivative containing platinum with a high affinity for the iodized ethyl ester of fatty acids of poppyseed oil (Lipiodol Ultra-fluide; Laboratoire Guerbet, Aulnay-Sous-Bois, France) that is used in TACE. Clinical trials have demonstrated that miriplatin is effective in the treatment of HCC.[14-19]

In a Phase II HCC study, the plasma concentration of total platinum in patients receiving miriplatin was very low, and no severe renal toxicity caused by miriplatin injection was reported.[17] Here we present three cases of HCC with stage 4 chronic renal failure who received transcatheter arterial chemotherapy with miriplatin.[20]

CASE REPORTS

1. Case 1

A 72-year-old man with HCC, liver cirrhosis, and diabetic nephropathy had undergone RFA four times and TACE three times over 5 years. As shown in Fig. 1, a computed tomography (CT) scan of the liver revealed multiple HCCs (tumor size, 15 to 34 mm; tumor number, three; stage, T2N0M0). The serum creati-

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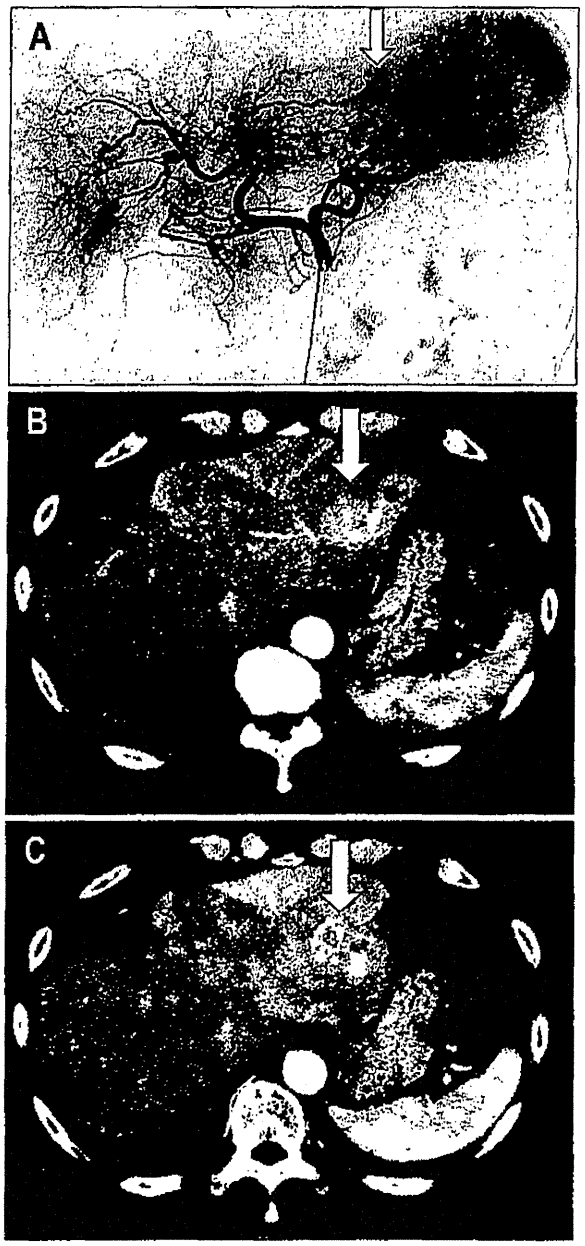

Fig. 1. Case 1. A 72-year-old man with unresectable hepatocellular carcinoma (HCC) who received transcatheter arterial chemoembolization (TACE) with miriplatin. (A) Abdominal angiography showed multiple HCCs (arrow). (B) Computed tomography (CT) showed multiple HCCs (arrow). (C) CT performed 1 month after TACE. The lesions revealed accumulations of lipiodol (arrow). Treatment efficacy was assessed as a partial response.

nine level was 2.3 mg/dL, and the estimated glomerular filtration rate (GFR) was 21.9 mL/min (Table 1).[21]

The patient was hydrated through a peripheral line. The femoral artery was catheterized under local anesthesia, and catheter was inserted superselectively into the hepatic artery that supplied the target tumor, for injection of the miriplatin/lipiodol suspension and 1 mm gelatin particles (1 mm-Gelpart; Nippon Kayaku, Tokyo, Japan). Miriplatin/lipiodol suspension was administrated slowly under careful fluoroscopic guidance. The dose of miriplatin/lipiodol was determined according to tumor size and the degree of liver dysfunction. The patient received TACE with miriplatin (miriplatin 50 mg, lipiodol 2.5 mL, and 1 mm-Gelpart were injected from both the right and left hepatic arteries). Therapy was well tolerated, and the patient's weight and serum creatinine level remained stable after treatment (Fig. 2). Major side effects included grade 1 fever, elevated blood glucose, and grade 1 nausea, which all resolved within 1 week (the National Cancer Institute's Common Terminology Criteria for Adverse Events [CTCAE] version 4.0). Treatment efficacy was assessed 1 month after treatment. Partial response (modified response evaluation criteria in solid tumors, mRECIST) was achieved in all target lesions.[22]

The patient was received two times TACE with miriplatin at intervals of 4 months after the first administration (second and third dosage of miriplatin were 120 mg and dosage of lipiodol were 6 mL). The patient's weight and serum creatinine level still remained stable after repeat injection of miriplatin (serum creatinine level was 2.2 mg/dL after third TACE with miriplatin). Stable disease (mRECIST) was achieved in all target lesions after third TACE with miriplatin.

2. Case 2

An 84-year-old man with HCC, liver cirrhosis, and chronic renal failure had undergone RFA three times and TACE six times over 10 years. As shown in Fig. 3, a CT scan of the liver showed multiple HCCs (tumor size, 12 to 55 mm; tumor number, six; stage, T3N0M0). The serum creatinine level was 1.6 mg/dL, and the estimated GFR was 20.3 mL/min (Table 1).

The patient was hydrated through a peripheral line. The femoral artery was catheterized under local anesthesia, and catheter was inserted superselectively into the hepatic artery that supplied the target tumor, for injection of the miriplatin/lipiodol suspension. Miriplatin/lipiodol suspension was administrated slowly under careful fluoroscopic guidance. The dose of miriplatin/lipiodol was determined according to tumor size and the degree of liver dysfunction.

The patient received transcatheter arterial chemotherapy with miriplatin (miriplatin 50 mg and lipiodol 2.5 mL were injected from both the right and left hepatic arteries). Therapy was well tolerated, and the patient's weight and serum creatinine level remained stable after treatment (Fig. 2). The major side effect of treatment was grade 1 fever, which resolved within 1 week (CTCAE version 4.0). Treatment efficacy was assessed 2 months after therapy. Stable disease (mRECIST) was achieved in all target lesions.

3. Case 3

An 83-year-old man with HCC, liver cirrhosis, hypertension,

Table 1. Patient Characteristics

Characteristic	Case 1	Case 2	Case 3
Age	72	84	83
Gender	Male	Male	Male
Height, cm	159	160	162
Weight, kg	58	47	57
Serum creatinine, mg/dL*	2.3	1.6	1.9
Estimated GFR1, mL/min†	21.9	20.3	22.2
Estimated GFR2, mL/min‡	22.8	32.5	27.0
Etiology	HCV	HCV	HBV
Child-Pugh score	A (6)	A (5)	A (5)
ICG-R15, %	16	13	4
Underlying disease that caused renal failure	Diabetic nephropathy	Chronic glomerulonephritis	Cisplatin induced renal failure
Tumor no.	3	6	40
Maximum tumor size, mm	34	55	39
Cancer stage (TNM)	II (T2N0M0)	III (T3N0M0)	II (T2N0M0)
Dosage of miriplatin, mg	100	100	70
Dosage of lipiodol, mL	5	5	3.5
Use of gelatin sponge particles	Yes	No	Yes
Contrast medium, mL	Iomeprol 60	Iomeprol 50	Iomeprol 190
Use of hydration therapy after miriplatin infusion	Yes	Yes	Yes

GFR, glomerular filtration rate; HCV, hepatitis C virus; HBV, hepatitis B virus; ICG-R15, indocyanine green retention rate at 15 minutes.
*Enzymatic method; †Cockcroft and Gault formula; ‡Japanese equation for estimating GFR.

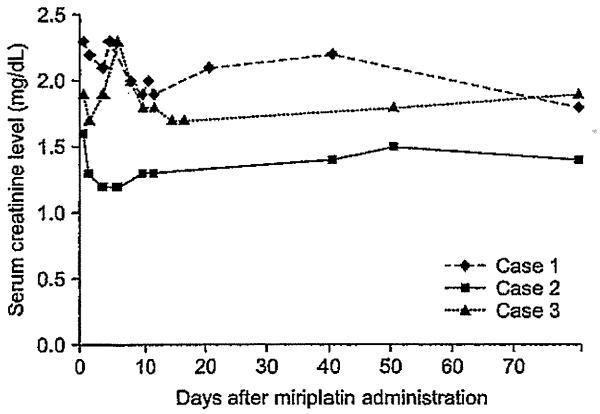

Fig. 2. Serum creatinine level after miriplatin administration in the three cases.

and renal failure that had been caused by cisplatin administration had undergone TACE nine times over 4 years. As shown in Fig. 4, a magnetic resonance imaging scan of the liver revealed multiple HCCs (tumor size, 5 to 39 mm; tumor number, 40; stage, T2N0M0). The patient's serum creatinine level was 1.9 mg/dL, and the estimated GFR was 22.2 mL/min (Table 1).

The patient was hydrated through a peripheral line. The femoral artery was catheterized under local anesthesia, and catheter was inserted superselectively into the hepatic artery that supplied the target tumor, for injection of the miriplatin/lipiodol suspension and 1 mm-Gelpart. Miriplatin/lipiodol suspension was administrated slowly under careful fluoroscopic guidance. The dose of miriplatin/lipiodol was determined according to tumor size and the degree of liver dysfunction.

The patient received TACE with miriplatin (miriplatin 30 mg, lipiodol 1.5 mL, and 1 mm-Gelpart were injected from the right and left hepatic arteries, and miriplatin 10 mg and lipiodol 0.5 mL were injected from the right inferior phrenic artery). Therapy was well tolerated, and the patient's weight and serum creatinine level remained stable after treatment (Fig. 2). Major side effects included grade 1 fever and grade 1 nausea, both of which resolved within 1 week (CTCAE version 4.0). Treatment efficacy was assessed 3 months after therapy. Stable disease (mRECIST) was achieved in all target lesions.

DISCUSSION

Various anticancer drugs, such as doxorubicin hydrochloride, epirubicin hydrochloride, mytomycin C, cisplatin, and neocarzinostatin, have been used at TACE agents for the treatment of HCC. However, the most effective and least toxic TACE protocol for HCC has yet to be identified.

Miriplatin is a novel lipophilic cisplatin derivative that can be suspended in lipiodol and used for transcatheter arterial che-

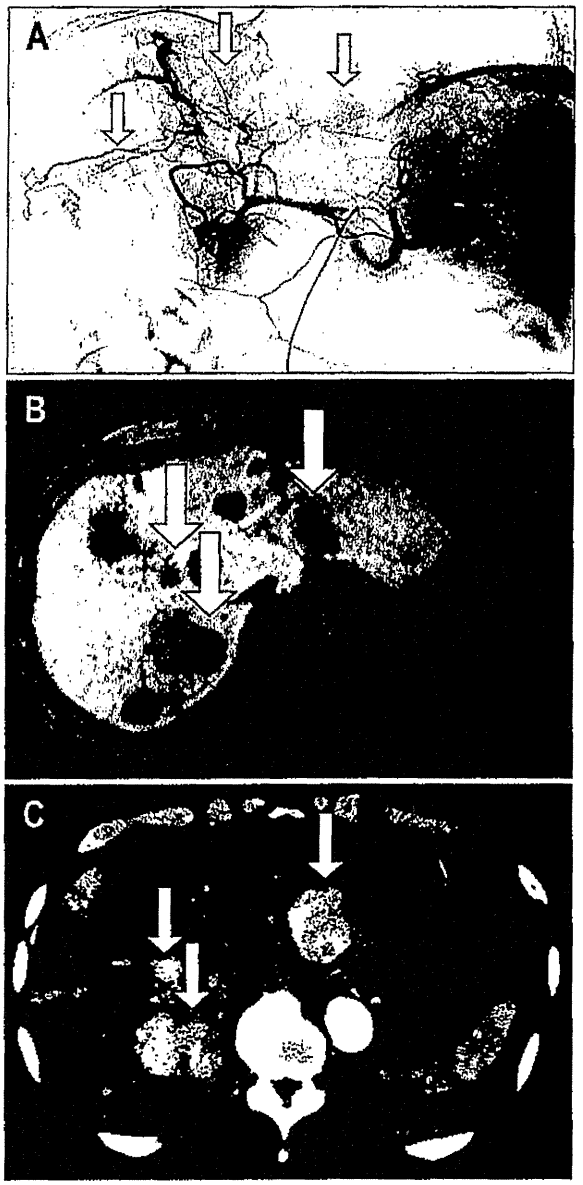

Fig. 3. Case 2. An 84-year-old man with unresectable hepatocellular carcinoma (HCC) who received transcatheter arterial chemotherapy with miriplatin. (A) Abdominal angiography showed multiple HCCs (arrows). (B) Magnetic resonance imaging (hepatobiliary phase) showed multiple HCCs (arrows). (C) Computed tomography performed 2 months after transcatheter arterial chemotherapy with miriplatin. The lesions showed accumulations of lipiodol (arrows). The treatment efficacy was assessed as a stable disease.

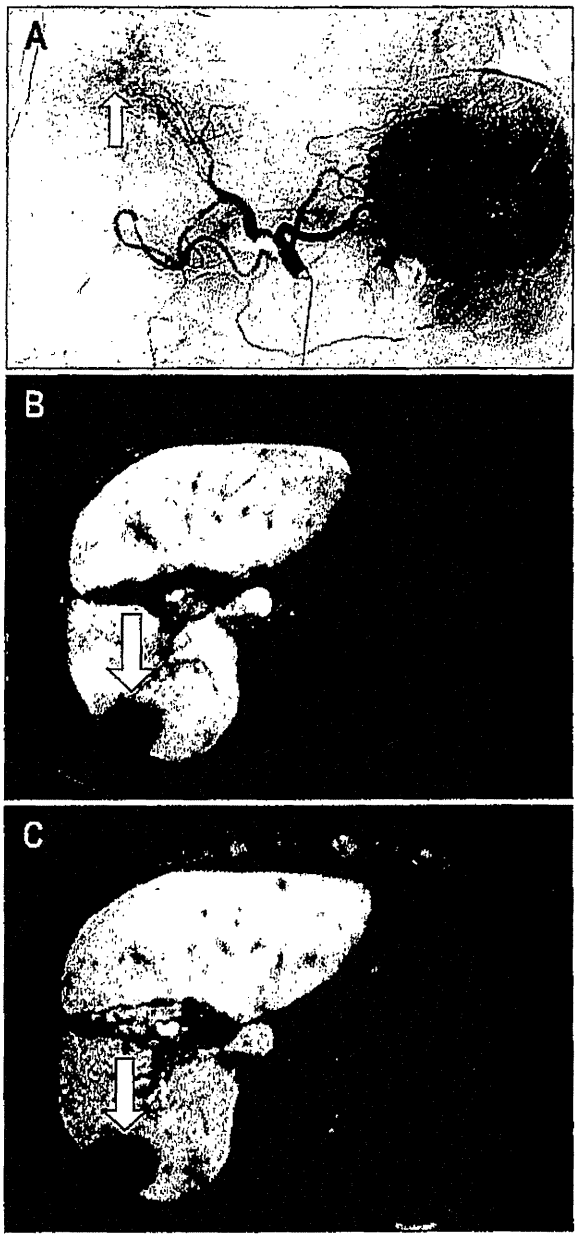

Fig. 4. Case 3. An 83-year-old man with unresectable hepatocellular carcinoma (HCC) who received transcatheter arterial chemoembolization (TACE) with miriplatin. (A) Abdominal angiography showed multiple HCCs (arrow). (B) Gadolinium ethoxybenzyl diethylenetriamine pentaacetic acid (Gd-EOB-DTPA) enhanced magnetic resonance imaging (MRI; hepatobiliary phase) showed multiple HCCs (arrow). (C) Gd-EOB-DTPA enhanced MRI performed 3 months after TACE. The lesions showed accumulations of lipiodol (arrow). The treatment efficacy was assessed as a stable disease.

motherapy of advanced HCC. It is one of the platinum agents, although hydration after administration is not necessary of its weak renal toxicity.

Various types of resistance to therapy can occur during repetition of TACE. Platinum derivatives are frequently administered to patients with advanced HCC that is unresponsive to anthracycline and antibiotic drugs.[23]

Miriplatin was developed as a lipophilic platinum complex in an effort to produce a superior antitumor effect in HCC with lower toxicity compared to cisplatin. Miriplatin-lipiodol suspension is a stable colloidal emulsion that is deposited within HCC tumors, where it gradually releases active derivatives of miripla-

tin.

According to pharmacokinetic studies, the plasma concentration of total platinum in patients treated with miriplatin is much lower than that after administration in patients administered intra-arterial cisplatin: the Cmax is approximately 300-fold lower and the Tmax roughly 500-fold longer than the corresponding values for intra-arterial cisplatin.[17] Theoretically, therefore, it can be administered even in patients of advanced HCC patients with chronic renal failure if visceral angiography can be performed.

Clinical trials have shown that miriplatin is effective for the treatment of HCC, but the safety and efficacy of miriplatin has not been evaluated in HCC patients with chronic renal failure.[16,17] Herein we presented three HCC cases with stage 4 chronic renal failure who received transcatheter arterial chemotherapy with miriplatin. In all three cases, no serious adverse events were observed, and serum creatinine level did not increase, even in the patient who had experienced renal failure due to cisplatin administration (Fig. 2). Repeated injection of miriplatin appears to be also safe in HCC patients with chronic renal failure.

The present results might suggest that transcatheter arterial chemotherapy with miriplatin can be safely used in HCC patients with chronic renal failure. A prospective study is required to assess the most effective, least nephrotoxic anticancer agent among the various platinum derivatives. Miriplatin appears to be a promising agent for HCC patients with chronic renal failure.

CONFLICTS OF INTEREST

The following authors have received honoraria (lecture fee) from Dainippon Sumitomo Pharma Co., Ltd., Osaka, Japan; Hiromitsu Kumada, MD, Kenji Ikeda, MD, Yasuji Arase, MD, Yoshiyuki Suzuki, MD, Fumitaka Suzuki, MD, and Norio Akuta, MD.

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