

## Clearance of hepatitis B surface antigen during long-term nucleot(s)ide analog treatment in chronic hepatitis B: results from a nine-year longitudinal study

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### Abstract

**Background** Clearance of hepatitis B surface antigen (HBsAg) is considered the ultimate goal in chronic hepatitis B treatment. One treatment option is long-term nucleot(s)ide analog (NA) therapy. We followed a group of long-term NA therapy patients to evaluate the efficacy of this treatment in promoting clearance and longitudinal declines of HBsAg.

**Method** The study included 791 NA therapy patients who received lamivudine as their first drug. At the baseline, 442 patients were hepatitis B e antigen (HBeAg)+ and 349 were HBeAg-. All analyses were performed after separating the HBeAg+ and HBeAg- cohorts. Cox proportional hazards models were used to determine which factors were associated with HBsAg clearance.

**Results** HBsAg clearance was observed in 18 (4.1 %) of the HBeAg+ patients and 20 (5.7 %) of the HBeAg- patients at baseline, giving seroclearance rates of 6.4 and 6.9 %, respectively, over the nine-year study period. HBsAg clearance was influenced by several independent factors that varied according to HBeAg cohort. For HBeAg+ patients, these included previous interferon therapy, infection with hepatitis B virus (HBV) genotype A, a  $\geq 0.5$  log IU/mL decline in HBsAg level within six months, and clearance of HBeAg at six months. For

HBeAg- patients, these included infection with HBV genotype A, decline in HBsAg at six months, and a baseline HBsAg level of  $< 730$  IU/mL.

**Conclusion** This study suggests that both direct antiviral potential and host immune response are needed to achieve HBsAg clearance by NA therapy. Viral genotype strongly influenced HBsAg clearance during NA therapy.

**Keywords** Hepatitis B surface antigen · Nucleot(s)ide analog · Lamivudine · Interferon

### Introduction

Worldwide, an estimated 400 million people are infected with hepatitis B virus (HBV) persistently, and one million people die of decompensated cirrhosis and/or hepatocellular carcinoma (HCC) annually [1, 2]. Recently, oral nucleot(s)ide analogs (NAs) have been used as a mainstay therapeutic strategy against chronic hepatitis B. Five such antiviral agents—lamivudine (LAM), entecavir (ETV), telbivudine, adefovir dipivoxil (ADV), and tenofovir disoproxil fumarate—which inhibit viral replication [e.g., hepatitis B virus DNA (HBV DNA) priming, reverse transcription of negative-stranded HBV DNA, and synthesis of positive-stranded HBV DNA] have been approved; these NAs vary in both the strength and the rapidity with which they suppress HBV DNA [3–10]. Sustained viral suppression by NA therapy can improve liver fibrosis and clinical outcomes of patients [11, 12]. LAM was the first NA to be approved to treat chronic hepatitis B in Japan, followed by ADV and ETV.

Responses to antiviral treatments can be evaluated by monitoring serum HBV DNA levels, hepatitis B e antigen (HBeAg) and antibody levels, and hepatitis B surface

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### Antiviral therapy and drug resistance

All 791 patients received 100 mg LAM daily as an initial therapy, but a LAM-resistant rtM204I/V mutation developed in 439 (55 %) of these patients. Over time, 334 (42 %) individuals experienced an increase in HBV DNA ( $\geq 1$  log copies/mL) [e.g., virological breakthrough (VBT)] and, as a result, 299 (98.5 %) individuals were also provided with ADV treatment (10 mg) added onto LAM as a rescue therapy. The remaining patients continued to receive LAM monotherapy and were lost to follow-up before the administration of ADV because of the lack of approval for ADV administration in Japan at the time. The resistant mutation for rtM204I/V was detected in 312 of 334 patients who experienced VBT using a commercial kit (as described below). Patients who had achieved an optimal or suboptimal virological response or who wished to participate in the clinical trial of ETV for LAM-refractory patients (ClinicalTrials.gov: NCT 1037166)—152 and 17 patients, respectively—switched from LAM to ETV (0.5 mg/day). Additionally, patients in whom subsequent ADV- or ETV-resistant mutants emerged received an optimal rescue therapy with other NAs (ETV + ADV combination for ADV resistance, and LAM + ADV combination for ETV resistance).

NA treatment was continued as a rule; median NA treatment duration was 75 months (25th–75th percentile, 55–102) in the HBeAg+ cohort and 92 months (67–119) in the HBeAg– cohort. Ultimately, 55 (7 %) of the 791 patients discontinued treatment; 16 of these individuals terminated treatment after achieving HBsAg seroclearance. Follow-ups were conducted for all patients, regardless of length of treatment, for as long as possible.

### Clinical data collection and follow-ups

Data on patient characteristics, biochemistry, hematology, virology, histology, and previous treatments were collected and registered in our institute's database at the time of patient enrollment. Prior to beginning LAM, all patients were surveyed about the presence of a family history of HBV infection. Data on treatment dose and duration of previous IFN therapy were collected from our hospital's IFN therapy database or requested from other hospitals as necessary. Complete details on the previous treatment were lacking for 29 (9.7 %) of 297 patients who received IFN therapy before starting LAM.

At least every 1–3 months, liver function and virological markers of HBV infection were measured in all patients. All serum HBsAg titers were measured from frozen serum samples collected at six months, one year, three years, five years, and once annually for 6–10 years, and then stored at  $-80^{\circ}\text{C}$ . The day of HBsAg clearance

was defined by the measurement in consecutive available serum samples before it was undetected in subsequent samples. A genotypic analysis of drug resistance was performed in cases of insufficient virological response or VBT, defined as an increase in serum HBV DNA levels  $\geq 1$  log above the nadir measured after the initial virological response. Cirrhosis was diagnosed by laparoscopy, liver biopsy, or clinical data such as imaging modalities and portal hypertension. The primary outcome for this study was HBsAg clearance. The endpoint of the follow-up was HBsAg clearance or last visit before January 2011.

### Markers of HBV infection

Serum HBsAg titers were measured 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 range 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 as the product document described. 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), which has a dynamic range of 2.6–7.6 log copies/mL, or COBAS TaqMan HBV v.2.0 (Roche Diagnostics, Tokyo, Japan), which has a dynamic range of 2.1–9.0 log copies/mL. A commercial kit (HBV Genotype EIA; Institute of Immunology) was used to serologically determine HBV genotypes using the combination of epitopes expressed on the pre-S2 region product, which is specific to each of the seven major genotypes (A–G). YMDD mutants (rt M204I/V) were determined by polymerase chain reaction-based enzyme-linked mini-sequence assay with a commercial kit (Genome Science Laboratories, Tokyo, Japan).

### Statistical analyses

Categorical data were compared between groups using chi-square or Fisher's exact tests. Continuous variables with a nonparametric distribution were analyzed with Mann–Whitney *U* tests, while those with a parametric distribution were analyzed with Student's *t* tests. When appropriate, Kruskal–Wallis tests were used to conduct pairwise comparisons of specific variables. Cox regression analyses were used to assess which variables were significantly associated with HBsAg clearance. Cut-off values were provided using the area under the receiver operating characteristic curve (ROC) only after rejecting the null hypothesis for the ROC curve. All baseline factors that were found to be significantly associated with HBsAg clearance by univariate analysis

antigen (HBsAg) and antibody levels. Serum HBsAg levels appear to reflect the amount of intrahepatic covalently closed circular DNA (cccDNA), which acts as a template for the transcription of viral genes [13–15]. Previous studies have shown that both interferon (IFN) and NA therapy result in a reduction of intrahepatic cccDNA [16, 17], suggesting that these treatments may be helpful in achieving the ultimate therapeutic goal of antiviral therapy for chronic hepatitis B (i.e., total clearance of HBsAg).

Very low rates of HBsAg clearance have been reported in the past [18–22]. Recent work has shown that over a one-year period, pegylated (PEG)-IFN therapy is more successful than ETV at reducing serum HBsAg [23]; furthermore, PEG-IFN therapy has also been reported to promote the complete clearance of HBsAg [24–27]. Several studies have detailed similar successes achieved by NA therapy but over relatively short (<5 years) treatment durations [18–20, 22, 28, 29]. The kinetics of HBsAg during long-term (>5 years) treatment remain unknown. NA therapy leads to time-dependent decreases in intrahepatic cccDNA and serum HBsAg levels if sustained viral suppression is longer term, and may therefore increase the rates of HBsAg clearance.

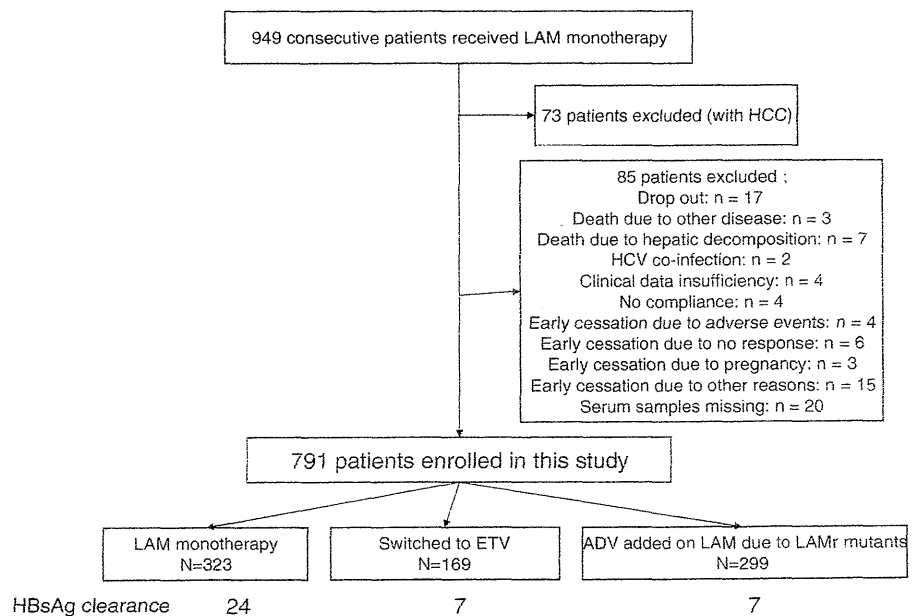
In order to evaluate this possibility empirically, we conducted a ten-year-long study in which we followed patients who received NA therapy initiated by the administration of LAM. We evaluated the resulting clearance and longitudinal declines of HBsAg using highly sensitive assays. Our aim was to determine whether long-term NA therapy can lead to HBsAg clearance, as suggested; if so, we also wished to elucidate the factors associated with its success.

**Methods**

**Study population**

Over a period of 12 years (September 1995 to September 2007), 949 consecutive patients who were chronically monoinfected with HBV (confirmed HBsAg positivity for at least six months), were treated with LAM monotherapy at the Department of Hepatology, Toranomon Hospital, Metropolitan Tokyo. The indication for antiviral therapy was abnormal ALT levels accompanying the increase in HBV DNA (over 4 log copies/mL) as a rule. However, in cases where ALT levels were normal, patients with advanced fibrosis were administered LAM. We did not treat patients without fibrosis who had low HBV DNA and normal ALT levels as a rule. We selected 791 patients for the final study after we had excluded all those who had been treated with LAM for <6 months, were co-infected with hepatitis C virus, had not provided sufficient serum samples, and/or had insufficient clinical records (Fig. 1). No patient was co-infected with human immunodeficiency virus in this cohort. Seven hundred ninety-one patients were enrolled in this cohort study. Of these 791 patients, 442 were HBeAg+ and 349 were HBeAg– at baseline. All analyses were performed after separating the HBeAg+ and HBeAg– cohorts. 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. This study has been registered in the University Hospital Medical Information Network Clinical Trials Registry (UMIN CTR) as the number UMIN000007993.

**Fig. 1** Schematic of study protocol. LAM lamivudine, HCC hepatocellular carcinoma, HCV hepatitis C virus, ETV entecavir, ADV adefovir dipivoxil, HBsAg hepatitis B surface antigen



were entered into a multivariate analysis. Independent baseline factors associated with clearance of HBsAg were calculated using a stepwise Cox regression analysis. We then performed a time-dependent Cox regression to analyze independent factors associated with HBsAg while adjusting for on-treatment factors and independent baseline factors. Three covariates of the on-treatment response factors—emergence of rtM204I/V mutants, VBT, and biochemical breakthrough—were set as the time-dependent covariates. Cumulative HBsAg clearance rates were analyzed using the Kaplan–Meier method; differences in the resulting curves were tested using log-rank tests. We performed Cox regression analysis, Kaplan–Meier curve analysis, and HBsAg kinetics analysis for no more than nine years, as the number of patients with a long-term follow-up of over ten years was too small to permit analysis [30]. Bonferroni adjustments were used to correct for the number of different ways a single predictor variable can be split. Significance was defined as  $P < 0.05$  for all two-tailed tests. Data analysis was performed with IBM SPSS version 19.0 software (IBM Corp., Armonk, NY, USA).

## Results

### Patient characteristics

Thirty-eight (4.8 %) of 791 patients successfully cleared HBsAg. Of these, 24 had received LAM, 7 had switched to ETV treatment, and 7 had been treated with both LAM and ADV (Fig. 1). Of the 38 patients who achieved HBsAg clearance, 18 were HBeAg+, whereas 20 were HBeAg– at baseline. Table 1 provides a comparison of the baseline and on-treatment characteristics between patients who were and were not able to successfully clear HBsAg (all patients, HBeAg+ and – cohorts, respectively). In the HBeAg+ cohort, baseline characteristics that were significantly associated with HBsAg clearance included previous IFN therapy, HBV genotype, HBV DNA, and AST and ALT levels; in the HBeAg– cohort, significant characteristics included HBV genotype and HBsAg levels. Significant on-treatment characteristics in the HBeAg+ cohort included decline in HBsAg, clearance of HBeAg, and decline in HBV DNA to  $<2.6$  log copies/mL at six months;

**Table 1** Baseline, demographic, and on-treatment characteristics of patients with and without HBsAg seroclearance

| Characteristics  | All patients<br>( <i>n</i> = 791) | HBeAg+ at baseline ( <i>n</i> = 442)        |  |                  | HBeAg– at baseline ( <i>n</i> = 349)        |  |                  |
|--|-----------------------------------|---|--|------------------|---|--|------------------|
|  |                                   | Persistently<br>HBeAg+<br>( <i>n</i> = 424) | HBsAg<br>seroclearance<br>( <i>n</i> = 18) | <i>P</i>         | Persistently<br>HBeAg+<br>( <i>n</i> = 329) | HBsAg<br>seroclearance<br>( <i>n</i> = 20) | <i>P</i>         |
| Baseline   |                                   |   |  |                  |   |  |                  |
| Age <sup>a</sup> (years) (SD)  | 43 (11.1)                         | 41 (11.2)                                   | 44 (10.5)                                  | 0.177            | 47 (10.3)                                   | 46 (10.3)                                  | 0.899            |
| Gender (male:female)   | 627:164                           | 329:95                                      | 16:2                                       | 0.385            | 265:64                                      | 16:4                                       | 1.000            |
| Race   |                                   |   |  | 0.446            |   |  |                  |
| Japanese   | 768 (97)                          | 411 (97)                                    | 17 (94)                                    |                  | 320 (97)                                    | 20 (100)                                   | 1.000            |
| Non-Japanese (%)<br>(Asian:Caucasian)                                | 23 (3) (21:2)                     | 13 (3) (20:2)                               | 1 (3) (1:0)                                |                  | 9 (3) (20:2)                                | 0 (3) (1:0)                                |                  |
| Family history of<br>HBV infection                                   | 539 (68)                          | 311 (73)                                    | 10 (56)                                    | 0.107            | 208 (63)                                    | 10 (50)                                    | 0.238            |
| Previous IFN therapy   | 297 (38)                          | 167 (39)                                    | 15 (83)                                    | <b>&lt;0.001</b> | 106 (32)                                    | 9 (45)                                     | 0.326            |
| IFN duration<br>(weeks)  | 27 (20–58)                        | 26 (18–53)                                  | 52 (21–79)                                 | 0.214            | 32 (22–89)                                  | 23 (14–72)                                 | 0.457            |
| Duration from the<br>end of IFN to<br>start of lamivudine<br>(weeks) | 50 (3–189)                        | 26 (7–124)                                  | 37 (2–89)                                  | 0.505            | 119 (3–316)                                 | 102 (18–289)                               | 0.746            |
| Previous NA<br>therapy   | 34 (4)                            | 21 (5)                                      | 2 (11)                                     | 0.239            | 10 (3)                                      | 1 (5)                                      | 0.483            |
| Presence of cirrhosis  | 169 (21)                          | 76 (18)                                     | 2 (11)                                     | 0.752            | 87 (26)                                     | 4 (20)                                     | 0.610            |
| HBV genotype   |                                   |   |  | <b>&lt;0.001</b> |   |  | <b>&lt;0.001</b> |
| A  | 28 (3.5)                          | 14 (3.3)                                    | 6 (33)                                     |                  | 6 (1.8)                                     | 2 (10)                                     |                  |
| B  | 67 (8.5)                          | 16 (3.8)                                    | 0 (0)                                      |                  | 48 (14.6)                                   | 3 (15)                                     |                  |
| C  | 664 (83.9)                        | 374 (88.2)                                  | 12 (67)                                    |                  | 265 (80.5)                                  | 13 (65)                                    |                  |
| D  | 3 (0.4)                           | 2 (0.4)                                     | 0 (0)                                      |                  | 0 (0)                                       | 1 (5)                                      |                  |
| F  | 2 (0.3)                           | 2 (0.4)                                     | 0 (0)                                      |                  | 0 (0)                                       | 0 (0)                                      |                  |
| Unclassified/missing   | 27 (3.4)                          | 16 (3.8)                                    | 0 (0)                                      |                  | 10 (3.0)                                    | 1 (5)                                      |                  |

**Table 1** continued

| Characteristics   | All patients<br>(n = 791) | HBeAg+ at baseline (n = 442)        |                                    |                  | HBeAg- at baseline (n = 349)        |                                    |                  |
|---|---------------------------|-------------------------------------|------------------------------------|------------------|-------------------------------------|------------------------------------|------------------|
|   |                           | Persistently<br>HBeAg+<br>(n = 424) | HBsAg<br>seroclearance<br>(n = 18) | <i>P</i>         | Persistently<br>HBeAg+<br>(n = 329) | HBsAg<br>seroclearance<br>(n = 20) | <i>P</i>         |
| Baseline HBV DNA<br>(log copies/mL)                                     | 7.0 (5.8–8.0)             | 7.6 (6.7–8.2)                       | 8.0 (7.5–8.4)                      | <b>0.027</b>     | 6.3 (5.2–7.2)                       | 6.1 (5.0–7.0)                      | 0.652            |
| Baseline HBsAg<br>level (IU/mL)   | 2530<br>(907–6590)        | 3910<br>(1690–12300)                | 5280<br>(943–67600)                | 0.331            | 1590 (599–3050)                     | 529 (58–1610)                      | <b>0.004</b>     |
| Baseline AST<br>level (IU/L)  | 74 (48–135)               | 81 (52–165)                         | 201 (78–666)                       | <b>0.011</b>     | 66 (42–113)                         | 57 (39–96)                         | 0.694            |
| Baseline AST<br>level (×ULN)  | 2.2 (1.5–4.1)             | 2.5 (1.6–5.0)                       | 6.1 (2.3–20.2)                     | <b>0.011</b>     | 2.0 (1.3–3.4)                       | 1.7 (1.2–2.9)                      | 0.736            |
| Baseline ALT<br>level (IU/L)  | 115<br>(63–252)           | 130 (72–290)                        | 326 (104–775)                      | <b>0.021</b>     | 101 (56–194)                        | 101 (55–215)                       | 0.904            |
| Baseline ALT<br>level (×ULN)  | 3.0 (1.7–6.4)             | 3.5 (1.9–7.8)                       | 7.8 (2.5–20.3)                     | <b>0.040</b>     | 2.6 (1.4–5.2)                       | 2.6 (1.4–5.2)                      | 0.955            |
| Baseline total<br>bilirubin level<br>(mg/dL)                            | 0.8 (0.6–1.1)             | 0.8 (0.5–1.1)                       | 0.9 (0.6–1.9)                      | 0.117            | 0.7 (0.6–1.0)                       | 0.8 (0.6–0.9)                      | 0.556            |
| Platelet count <sup>a</sup><br>(10 <sup>3</sup> /mm <sup>3</sup> ) (SD) | 16.1 (5.7)                | 16.5 (6.1)                          | 14.7 (3.5)                         | 0.221            | 15.6 (5.1)                          | 17.7 (6.9)                         | 0.216            |
| <b>On-treatment response</b>  |                           |                                     |                                    |                  |                                     |                                    |                  |
| Decline of HBsAg<br>level (≥0.5 log<br>IU/mL within<br>six months)      | 97 (1)                    | 67 (16)                             | 13 (72)                            | <b>&lt;0.001</b> | 11 (3)                              | 6 (30)                             | <b>&lt;0.001</b> |
| HBeAg<br>positive → clearance<br>within six months                      | 109 (14)                  | 94 (22)                             | 10 (56)                            | <b>0.005</b>     | NA                                  | NA                                 |                  |
| Undetectable HBV<br>DNA (<400 copies/<br>mL) at six months              | 532 (67)                  | 221 (52)                            | 15 (83)                            | <b>0.014</b>     | 277 (84)                            | 19 (95)                            | 0.330            |
| Emergence of<br>rtM204I/V mutants                                       | 439 (55)                  | 251 (59)                            | 9 (50)                             | 0.469            | 170 (52)                            | 9 (45)                             | 0.646            |
| Viral breakthrough<br>due to mutants                                    | 334 (42)                  | 216 (51)                            | 5 (28)                             | 0.055            | 108 (33)                            | 5 (25)                             | 0.473            |
| Biochemical<br>breakthrough<br>due to mutants                           | 318 (40)                  | 200 (47)                            | 5 (28)                             | 0.146            | 108 (33)                            | 5 (25)                             | 0.473            |

Except where marked with a superscript letter a, values are expressed as the median and 25th–75th percentiles (parenthetically), or number and percentage (parenthetically). ULN; AST = 33 IU/L, ALT = 42 IU/L (male), and 27 IU/L (female). *Asterisks* indicate data displayed as mean values and standard deviations. *Bold text* indicates statistically significant *P* values

the only significant characteristic in the HBeAg- cohort was a decline in HBsAg within six months. ROC curve analysis confirmed a cut-off value of 0.5 log IU/mL for a decline in HBsAg level within six months in the HBeAg+ and - cohorts [area under the curve = 0.810 (95 % CI 0.673–0.947) (HBeAg+ cohort) and 0.760 (95 % CI 0.611–0.909) (HBeAg- cohort)].

LAM-resistant rtM204I/V mutants were detected in 439 (55.5 %) of 791 patients. Of these, 334 (42.2 % of all patients) also developed VBT accompanied by an increase in HBV DNA (≥1 log copies/mL). The rate of VBT was

marginally significantly lower in the HBsAg clearance group in the HBeAg+ cohort (Table 1).

**Factors associated with HBsAg clearance**

The overall cumulative rates of HBsAg clearance were 0.2 % at one year, 1.2 % at three years, 2.6 % at five years, 4.2 % at seven years, and 6.4 % at nine years in the HBeAg+ cohort; and 0.6 % at one year, 0.9 % at three - years, 2.2 % at five years, 5.2 % at seven years, and 6.9 % at nine years in the HBeAg- cohort. Univariate Cox

**Table 2** Baseline and on-treatment response factors associated with HBsAg clearance, as determined by time-dependent univariate and multivariate analyses at year 9 (HBeAg+ cohort)

| Variable   | Univariate                           |                  | Multivariate                         |                  |
|--|--------------------------------------|------------------|--------------------------------------|------------------|
|  | HBsAg clearance rate ratio (95 % CI) | <i>P</i>         | HBsAg clearance rate ratio (95 % CI) | <i>P</i>         |
| <b>Baseline factors</b>  |                                      |                  |                                      |                  |
| Age ( $\geq 50$ years)   | 1.36 (0.48–3.86)                     | 0.564            |                                      |                  |
| Gender (F)   | 0.51 (0.12–2.23)                     | 0.371            |                                      |                  |
| Family history of HBV infection                                  | 0.42 (0.16–1.09)                     | 0.074            |                                      |                  |
| Previous IFN therapy   | <b>5.60 (1.61–19.5)</b>              | <b>0.007</b>     | <b>6.15 (1.69–22.4)</b>              | <b>0.006</b>     |
| Previous NA therapy  | 2.42 (0.55–10.6)                     | 0.242            |                                      |                  |
| Presence of cirrhosis  | 0.85 (0.52–1.40)                     | 0.527            |                                      |                  |
| HBV genotype (A)   | <b>3.64 (2.21–5.99)</b>              | <b>&lt;0.001</b> | <b>3.18 (1.80–5.62)</b>              | <b>&lt;0.001</b> |
| HBV DNA ( $\geq 6.0$ log copies/mL)                              | 2.56 (0.34–19.3)                     | 0.362            |                                      |                  |
| HBsAg ( $< 730$ IU/mL)   | 1.57 (0.51–4.81)                     | 0.432            |                                      |                  |
| AST ( $\geq 4.5 \times$ ULN)                                     | <b>4.53 (1.68–12.2)</b>              | <b>0.003</b>     |                                      |                  |
| ALT ( $\geq 7.2 \times$ ULN)                                     | <b>3.56 (1.35–9.36)</b>              | <b>0.010</b>     |                                      |                  |
| Total bilirubin ( $\geq 1.5$ mg/dL)                              | 2.63 (0.92–7.46)                     | 0.070            |                                      |                  |
| Platelet count ( $< 1.2 \times 10^3$ /mm <sup>3</sup> )          | 0.58 (0.13–2.59)                     | 0.476            |                                      |                  |
| <b>On-treatment response factors</b>                             |                                      |                  |                                      |                  |
| Decline of HBsAg level ( $\geq 0.5$ log IU/mL within six months) | <b>15.8 (5.14–48.5)</b>              | <b>&lt;0.001</b> | <b>18.6 (5.78–60.0)</b>              | <b>&lt;0.001</b> |
| HBeAg positive $\rightarrow$ clearance within six months         | <b>4.33 (1.65–11.4)</b>              | <b>0.003</b>     | <b>2.95 (1.04–8.39)</b>              | <b>0.042</b>     |
| Undetectable HBV DNA ( $< 400$ copies/mL) at six months          | <b>3.95 (1.14–13.7)</b>              | <b>0.031</b>     |                                      |                  |
| Emergence of rtM204I/V mutants <sup>a</sup>                      | 0.88 (0.32–2.44)                     | 0.802            |                                      |                  |
| Viral breakthrough due to mutants <sup>a</sup>                   | <b>0.32 (0.10–1.00)</b>              | <b>0.050</b>     |                                      |                  |
| Breakthrough hepatitis due to mutants <sup>a</sup>               | 0.41 (0.13–1.31)                     | 0.134            |                                      |                  |

<sup>a</sup> Time-dependent covariates. *Bold text* indicates statically significant *P* values. Variables analyzed in multivariate analysis: previous IFN therapy, HBV genotype, ALT, decline of HBsAg levels, HBeAg clearance within six months, undetectable HBV DNA at six months, and viral breakthrough due to mutants (time-dependent covariate)

regression analysis identified four baseline characteristics and four on-treatment responses that were associated with HBsAg clearance in the HBeAg+ cohort (Table 2), and two baseline characteristics and two on-treatment responses in the HBeAg– cohort (Table 3). ROC curve analysis provided the optimal cut-off values and indices for the prediction of HBsAg clearance. ROC curve analysis confirmed cut-off indices of  $4.5 \times$  ULN for AST and  $7.2 \times$  ULN for ALT for HBsAg clearance in the HBeAg+ cohort [area under the curve = 0.677 (95 % CI 0.524–0.830) (AST) and 0.643 (95 % CI 0.503–0.783) (ALT)]. Meanwhile, ROC curve analysis confirmed a cut-off value of 730 IU/mL (2.86 log IU/mL) for HBsAg for HBsAg clearance in the HBeAg– cohort [area under the curve = 0.696 (95 % CI 0.556–0.836)]. Time-dependent multivariate Cox regression analysis identified two significant baseline characteristics and two on-treatment responses related to HBsAg clearance: previous IFN therapy, infection with HBV genotype A, a decline in HBsAg level of  $\geq 0.5$  log IU/mL within six months, and HBeAg clearance within six months in the HBeAg+ cohort (Table 2). In the HBeAg– cohort, two baseline characteristics and one on-treatment response

were identified in multivariate analysis: infection with HBV genotype A, HBsAg level of  $< 730$  IU/mL (2.86 log IU/mL), and a decline in HBsAg level of  $\geq 0.5$  log IU/mL within six months (Table 3).

#### Association between HBV genotype and HBsAg clearance

We performed a detailed analysis of the association between HBV genotype and HBsAg clearance in patients treated with NAs. Median baseline HBsAg levels were 4.7 log IU/mL (25th–75th percentile, 4.4–5.1) among patients with genotype A, 3.8 (3.5–4.2) among patients with genotype B, and 3.5 (3.2–4.0) among patients with genotype C in the HBeAg+ cohort (Fig. 2a); and 3.7 (2.5–4.1) in patients with genotype A, 2.9 (2.6–3.5) in patients with genotype B, and 3.2 (2.8–3.5) in patients with genotype C in the HBeAg– cohort (Fig. 2b). HBeAg+ patients with genotype A had higher baseline HBsAg levels than those with genotypes B or C ( $P < 0.001$ ) (Fig. 2a). There were no significant differences in baseline HBsAg levels between the genotypes in the HBeAg– cohort.

**Table 3** Baseline and on-treatment response factors associated with HBsAg clearance, as determined by time-dependent univariate and multivariate analyses at year 9 (HBeAg- cohort)

| Variable   | Univariate                           |                  | Multivariate                         |                  |
|--|--------------------------------------|------------------|--------------------------------------|------------------|
|  | HBsAg clearance rate ratio (95 % CI) | P                | HBsAg clearance rate ratio (95 % CI) | P                |
| <b>Baseline factors</b>  |                                      |                  |                                      |                  |
| Age ( $\geq 50$ years)   | 1.39 (0.54–3.60)                     | 0.498            |                                      |                  |
| Gender (F)   | 0.98 (0.28–3.40)                     | 0.971            |                                      |                  |
| Family history of HBV infection                                  | 0.49 (0.19–1.27)                     | 0.140            |                                      |                  |
| Previous IFN therapy   | 0.88 (0.32–2.38)                     | 0.797            |                                      |                  |
| Previous NA therapy  | 2.41 (0.32–18.2)                     | 0.394            |                                      |                  |
| Presence of cirrhosis  | 0.71 (0.43–1.16)                     | 0.173            |                                      |                  |
| HBV genotype (A)   | <b>2.79 (1.33–5.85)</b>              | <b>0.007</b>     | <b>2.73 (1.29–5.81)</b>              | <b>0.009</b>     |
| HBV DNA ( $\geq 6.0$ log copies/mL)                              | 1.16 (0.43–3.14)                     | 0.772            |                                      |                  |
| HBsAg ( $< 730$ IU/mL)   | <b>3.91 (1.59–9.52)</b>              | <b>0.003</b>     | <b>4.90 (1.85–10.6)</b>              | <b>0.001</b>     |
| AST ( $\geq 4.5 \times$ ULN)                                     | 1.76 (0.57–5.40)                     | 0.324            |                                      |                  |
| ALT ( $\geq 7.2 \times$ ULN)                                     | 1.89 (0.62–5.81)                     | 0.265            |                                      |                  |
| Total bilirubin ( $\geq 1.5$ mg/dL)                              | 1.18 (0.27–5.20)                     | 0.825            |                                      |                  |
| Platelet count ( $< 1.2 \times 10^5$ /mm <sup>3</sup> )          | 0.77 (0.17–3.55)                     | 0.733            |                                      |                  |
| <b>On-treatment response factors</b>                             |                                      |                  |                                      |                  |
| Decline of HBsAg level ( $\geq 0.5$ log IU/mL within six months) | <b>11.5 (4.24–31.0)</b>              | <b>&lt;0.001</b> | <b>16.9 (5.89–48.4)</b>              | <b>&lt;0.001</b> |
| Undetectable HBV DNA ( $< 400$ copies/mL) at six months          | 2.78 (0.37–20.8)                     | 0.322            |                                      |                  |
| Emergence of rtM204I/V mutants <sup>a</sup>                      | 0.64 (0.23–1.79)                     | 0.392            |                                      |                  |
| Viral breakthrough due to mutants <sup>a</sup>                   | 0.72 (0.23–2.29)                     | 0.581            |                                      |                  |
| Breakthrough hepatitis due to mutants <sup>a</sup>               | 0.65 (0.21–2.06)                     | 0.465            |                                      |                  |

<sup>a</sup> Time-dependent covariates. *Bold text* indicates statically significant *P* values

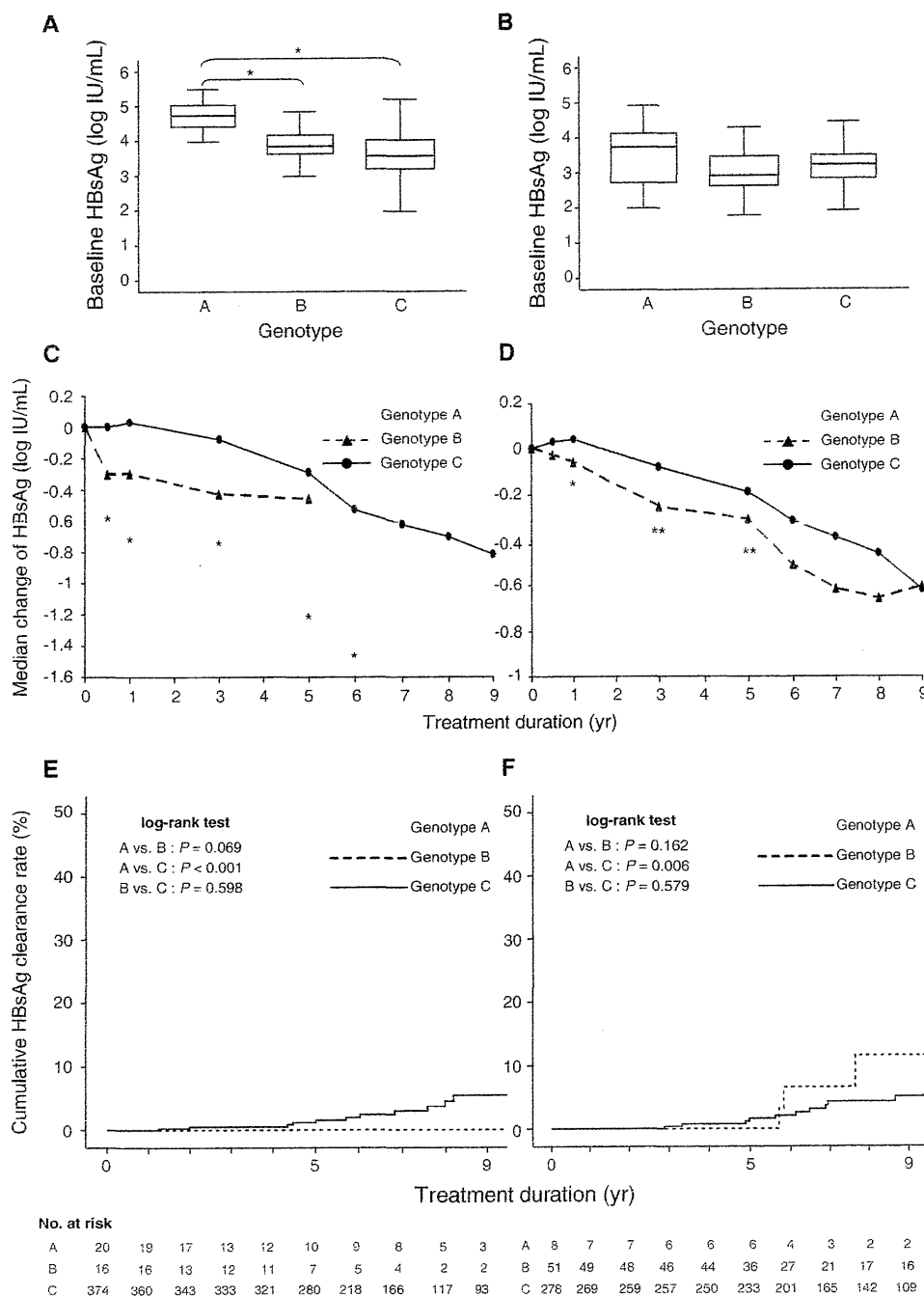
Variables analyzed in multivariate analysis: HBV genotype, baseline HBsAg, decline of HBsAg levels

HBsAg kinetics over time in the HBeAg+ and - cohorts are shown in Fig. 2c, d, respectively. Among patients with genotype A in the HBeAg+ cohort, the median HBsAg change from baseline was  $-0.44$  log IU/mL at six months,  $-0.56$  at one year,  $-0.58$  at three years,  $-1.08$  at five years, and  $-1.33$  at six years. Among patients with genotype B in the HBeAg+ cohort, median changes were  $-0.30$  log IU/mL at six months,  $-0.30$  at one year,  $-0.43$  at three years, and  $-0.46$  at five years. Kinetics were not calculated for some groups (genotype A at seven years, genotype B at six years) because the number of patients was too small. Finally, among patients with genotype C in the HBeAg+ cohort, median changes were  $0.00$  log IU/mL at six months,  $0.03$  at one year,  $-0.08$  at three years,  $-0.29$  at five years,  $-0.53$  at six years,  $-0.62$  at seven years,  $-0.70$  at eight years, and  $-0.82$  at nine years. Genotype had a significant effect on the slopes between data collection points at six months and six years. In the HBeAg+ cohort, declines were faster in patients with genotype A than in those with genotypes B or C. HBeAg- patients with genotype A displayed a median HBsAg change from baseline of  $0.05$  log IU/mL at six months,  $0.05$  at one year,  $-0.11$  at three years,  $-0.21$  at

five years, and  $-0.26$  at six years. Among patients with genotype B in the HBeAg- cohort, median changes were  $-0.03$  log IU/mL at six months,  $-0.06$  at one year,  $-0.25$  at three years,  $-0.31$  at five years,  $-0.51$  at six years,  $-0.62$  at seven years,  $-0.66$  at eight years, and  $-0.61$  at nine years. Among patients with genotype C in the HBeAg- cohort, median changes were  $0.03$  log IU/mL at six months,  $0.04$  at one year,  $-0.08$  at three years,  $-0.19$  at five years,  $-0.32$  at six years,  $-0.39$  at seven years,  $-0.46$  at eight years, and  $-0.62$  at nine years. The decline was slightly faster in patients with genotype B than in those with genotypes A and C in the HBeAg- cohort.

We investigated whether HBsAg clearance were influenced by genotype or baseline HBeAg. Cumulative HBsAg clearance rates in the HBeAg+ cohort were as follows:  $15\%$  at year 3, and  $35\%$  at year 5 in patients with genotype A;  $0\%$  over all years in patients with genotype B; and  $0.6\%$  at year 3,  $1.2\%$  at year 5, and  $5.4\%$  at year 9 in patients with genotype C (Fig. 2e). In the HBeAg- cohort, clearance rates were  $12\%$  at year 3, and  $25\%$  at year 5 in patients with genotype A;  $0\%$  at year 3,  $0\%$  at year 5, and  $11.5\%$  at year 9 in patients with genotype B; and  $0.4\%$  at year 3,  $1.6\%$  at year 5, and  $5.1\%$  at year 9 in

**Fig. 2** **a** Box plot of baseline HBsAg levels in patients with different HBV genotypes (HBeAg+ cohort). The asterisk (\*) indicates a statistical significance of  $P < 0.001$ , as determined by the Mann-Whitney  $U$  test and Bonferroni correction. **b** Box plot of baseline HBsAg levels in patients with different HBV genotypes (HBeAg- cohort). **c** Median change in HBsAg level from baseline in patients with different HBV genotypes (HBeAg+ cohort). A single asterisk (\*) indicates  $P < 0.001$ , as determined by the Kruskal-Wallis test. **d** Median change in HBsAg level from baseline in patients with different HBV genotypes (HBeAg- cohort). A single asterisk (\*) indicates  $P < 0.001$  and a double asterisk (\*\*) indicates  $P < 0.02$ , as determined by the Kruskal-Wallis test. **e** Kaplan-Meier life table showing cumulative HBsAg clearance rates in patients with different HBV genotypes (HBeAg+ cohort). Cumulative HBsAg clearance rates were significantly higher among patients with genotype A (log-rank test; A vs. B:  $P = 0.069$ , A vs. C:  $P < 0.001$ , B vs. C:  $P = 0.598$ , after Bonferroni correction). **f** Kaplan-Meier life table showing cumulative HBsAg clearance rates in patients with different HBV genotypes (HBeAg- cohort). Cumulative HBsAg clearance rates were significantly higher among patients with genotype A (log-rank test; A vs. B:  $P = 0.169$ , A vs. C:  $P = 0.006$ , B vs. C:  $P = 0.579$ , after Bonferroni correction)



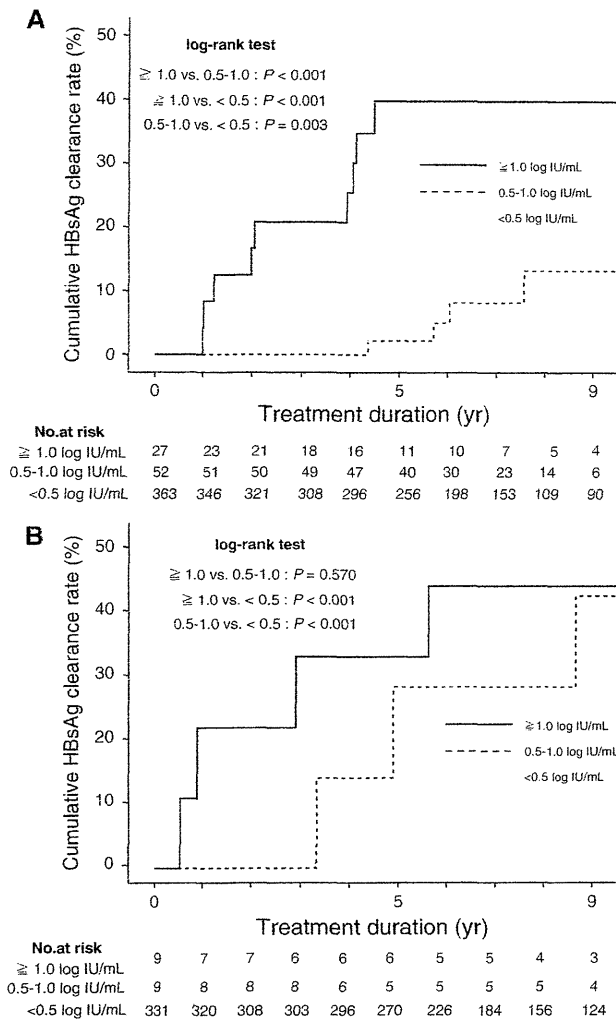
patients with genotype C (Fig. 2f). Clearance rates were significantly higher in patients with genotype A than in those with genotype C ( $P < 0.001$  in the HBeAg+ cohort,  $P = 0.006$  in the HBeAg- cohort).

Association between on-treatment response and subsequent HBsAg clearance

We stratified patients into three groups according to the amount of HBsAg decline within the first six months of

treatment; this allowed us to evaluate the impact of on-treatment response factors on the clearance of HBsAg. The stratifications were as follows: rapid decline ( $\geq 1.0$  log IU/mL), intermediate decline (0.5–1.0 log IU/mL), and slow decline or steady ( $< 0.5$  log IU/mL). Cumulative HBsAg clearance rates in the HBeAg+ cohort were 11 % at year 3, and 40 % at year 5 in the rapid decline group; 0 % at year 3, 2.2 % at year 5, and 13 % at year 9 in the intermediate decline group; and 0 % at year 3, 0 % at year 5, and 2.9 % at year 9 in the slow decline or steady group (Fig. 3a).





**Fig. 3** **a** Kaplan–Meier life table showing cumulative HBsAg clearance rates in patients with varying rates of HBsAg decline within the first six months (HBeAg+ cohort). Clearance rates were highest in the rapid decline group, followed by the intermediate decline group and the slow or steady group (log-rank test; rapid vs. intermediate:  $P < 0.001$ , rapid vs. slow:  $P < 0.001$ , intermediate vs. slow:  $P = 0.003$ , after Bonferroni correction). **b** Kaplan–Meier life table showing cumulative HBsAg clearance rates in patients with varying rates of HBsAg decline within the first six months (HBeAg– cohort). Clearance rates were highest in the rapid decline group, followed by the intermediate decline group and the slow or steady group (log-rank test; rapid vs. intermediate:  $P = 0.570$ , rapid vs. slow:  $P < 0.001$ , intermediate vs. slow:  $P < 0.001$ , after Bonferroni correction)

Cumulative HBsAg clearance rates in the HBeAg– cohort were 33 % at year 5, and 44 % at year 7 in the rapid decline group; 0 % at year 3, 29 % at year 5, and 43 % at year 9 in the intermediate decline group; and 0.3 % at year 3, 0.7 % at year 5, and 4.6 % at year 9 in the slow decline or steady group (Fig. 3b). Clearance rates were highest in the rapid decline group, followed by the intermediate decline group and the slow or steady group in both the

HBeAg+ and HBeAg– cohorts. The decline of HBsAg within the first six months was a strong predictor of HBsAg clearance.

### Viral breakthrough and subsequent HBsAg clearance

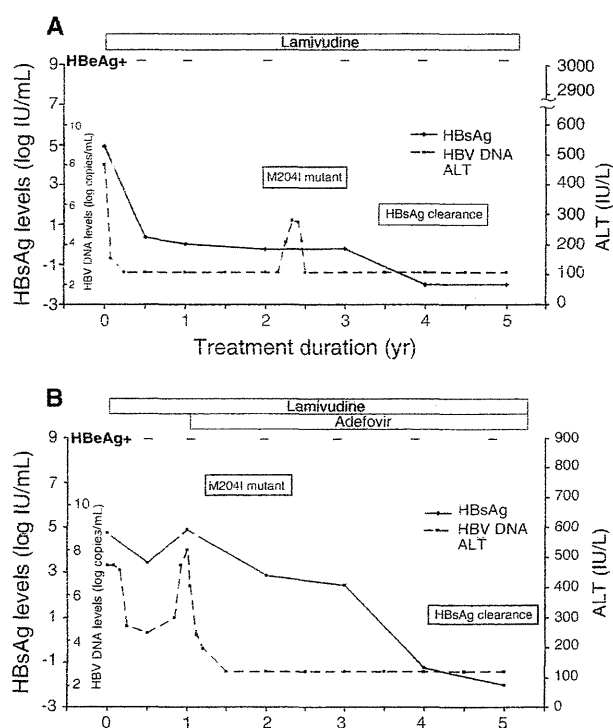
Although VBT was not associated with HBsAg clearance in the multivariate model, as described above, HBsAg clearance was observed in ten patients who experienced VBT (five patients in the HBeAg+ cohort and five in the HBeAg– cohort). All ten patients achieved clearance of HBsAg after VBT occurred. Six of these patients received ADV added on to LAM for VBT, and subsequently achieved clearance of HBsAg (five patients in the HBeAg+ cohort and one in the HBeAg– cohort). The other four patients spontaneously recovered from VBT while continuing to receive LAM monotherapy, and subsequently achieved clearance of HBsAg (one patient in the HBeAg+ cohort and three in the HBeAg– cohort). LAM-resistant mutant strains (M204I/V mutants) were detected in nine patients in whom VBT occurred. HBV DNA negativity continued for the follow-up period after HBsAg clearance in these ten patients. The typical clinical and virological courses of two representative patients who achieved HBsAg clearance after VBT are shown in Fig. 4a, b.

### Virological courses after discontinuation of NAs

Sixteen (42.1 %) of 38 patients with HBsAg clearance discontinued NA treatment due to HBsAg clearance. Median interval between HBsAg clearance and discontinuation of NAs was nine months (range 2–29 months). Median follow-up period after discontinuation of NAs was 24 months (range 7–171) in these patients. No relapses of serum HBsAg or HBV DNA were observed during the follow-up period. Serum anti-HBs appeared in 12 (75 %) of the 16 patients who discontinued NAs. Median time to the appearance of anti-HBs after HBsAg clearance was 16 months (range 2–92) in patients who discontinued NAs. Two of 22 patients who continued NAs with HBsAg clearance had the appearance of anti-HBs, and median time to the appearance of anti-HBs after HBsAg clearance was two and seven months in these two patients, respectively.

### Discussion

We found that three baseline factors and two on-treatment response factors are associated with HBsAg clearance in patients who begin treatment with LAM and continue with long-term NA therapy. HBV genotype and the decline in HBsAg over the first six months were associated with



**Fig. 4** Case presentation of the typical clinical and virological courses of two representative patients who achieved HBsAg clearance after VBT occurred. **a** Patient 1, a 45-year-old man who was HBeAg+ at baseline and had genotype A. **b** Patient 2, a 38-year-old man who was HBeAg+ at baseline and had genotype A. VBT virological breakthrough

HBsAg clearance in both the HBeAg+ and – cohorts, whereas the clearance of HBsAg was associated with previous IFN therapy and the clearance of HBeAg over the first six months only in the HBeAg+ cohort, and baseline HBsAg levels only in the HBeAg– cohort.

HBV genotype was recently reported to influence declines in and the clearance of HBsAg among patients who underwent PEG-IFN therapy [31]. In one study where negativity for serum HBV DNA and seroconversion of HBeAg represented the study end point, genotype was not found to influence response to NA therapy [31]. However, other reports have indicated that genotype does impact on declines in and the clearance of HBsAg [20, 29]. Heathcote et al. [20] reported that 20 HBeAg+ patients (8 %) who were treated with tenofovir achieved HBsAg clearance in three years. Twelve (60 %) of 20 patients were infected with genotype A and the others with genotype D. In this study, cumulative HBsAg clearance rates were 15 % at year 3 in HBeAg+ patients with genotype A. This result seems to be similar regardless of the antiviral potential. Previous studies with more ethnically diverse study populations than ours found that HBsAg clearance rates were highest in patients with genotype A. The similarity between

those results and ours implies that the HBV genotype is more influential than ethnicity on HBsAg clearance during NA therapy. Of 28 genotype A patients in our population, the majority (79 %) did not have a family history of infection. Recent work has shown that sexual transmission of acute HBV genotype A infections is increasing in Japan, resulting in chronic HBV infection, especially in young adult patients [32, 33]. Cumulatively, these findings imply that HBsAg clearance is more likely in genotype A patients because they have been infected with HBV for a shorter period of time. Furthermore, Hou et al. [34] demonstrated that genotype A responded better than other HBV genotypes to IFN therapy. They revealed that a lower number of amino acid substitutions at baseline were associated with a better response to IFN therapy, and that this variable was linked with HBV genotype A, which had the lowest number of amino acid substitutions in the core gene among genotypes B, C, or D. Although amino acid substitutions in the core gene were not analyzed in this study, the relation between the core gene and treatment responses of NAs is necessary to be investigated in the future.

Although Gish et al. [19] reported that previous IFN therapy is not associated with HBsAg clearance in patients who are HBeAg+, the opposite was true in our HBeAg+ cohort. These contradictory findings may result from the fact that their patients received NA therapy over a much shorter time period (median duration 23 vs. 75 months, a 3.2-fold difference). We believe that there are two main reasons why HBsAg clearance rates were higher in patients who had previously received IFN therapy: the influence of AST/ALT flares after IFN therapy and changes in host immune response to HBV as a result of the immunomodulating activity of IFN. It has previously been shown that in patients with high baseline ALT levels, HBV DNA and HBeAg are likely to rapidly decrease during NA therapy [35, 36]. In this study, HBsAg clearance was likely to occur in patients who had high ALT levels at baseline, and in patients with previous IFN therapy (Table 2) in the HBeAg+ cohort. High virological responses have been reported in response to robust ALT flares induced by IFN therapy [37, 38]. Moreover, Wursthorn et al. [29] recently indicated that the antiviral potential of NAs and antiviral T cell reactivity are associated with HBsAg clearance in response to telbivudine treatment. These findings may be also associated with the achievement of HBsAg clearance after VBT occurs. Taken together, these results imply that both direct antiviral potential and host immune response are needed to achieve HBsAg clearance, especially in HBeAg+ patients.

We found that the initial HBsAg reduction was a strong predictor of subsequent HBsAg clearance during NA therapy, which supports a similar previous finding [29]. HBsAg reduction over the initial six months is important

for predicting the subsequent HBsAg kinetics in both HBeAg+ and HBeAg- patients. The novel finding in this study was that HBeAg- individuals achieved HBsAg clearance. We found that the median duration to HBsAg clearance was longer in patients with HBeAg- than in those who were HBeAg+ in this study (6.0 vs. 4.4 years). Manesis et al. [28] used modeling to determine that HBeAg- patients receiving LAM treatment would likely require >10 years to achieve HBsAg loss. Furthermore, baseline HBsAg titers were <730 IU/mL in 60 % (12/20) of HBeAg- patients who achieved HBsAg clearance. The only baseline predictive factor of HBsAg clearance was baseline HBsAg levels in HBeAg- patients, except for genotype. There was no difference in HBsAg clearance rates in HBeAg- patients with high- and low-baseline HBV DNA or ALT levels. We hypothesize that HBsAg clearance in these patients may result from long treatment duration and low HBsAg titers.

Our study was limited by the fact that it was a hospital-based retrospective analysis, which means there may be some bias associated with patient type and treatment selection. We were unable to compare HBsAg clearance rates obtained in our study with those of controls untreated with NA. Because all subjects in the study received LAM as an initial NA, and then received rescue therapy when drug-resistant mutations emerged, NA therapy regimens were not uniform across all patients, and there were variations in both treatment dose and duration of previous IFN therapy. We were not able to collect immunological data on our subjects. Finally, our results need to be validated by further studies investigating a large study population receiving long-term ETV or tenofovir with high antiviral potential and a high genetic barrier.

Despite these drawbacks, we were able to determine several factors associated with HBsAg clearance, including HBV genotype and a decline in HBsAg over the initial six months of treatment (HBeAg+ and - cohorts); previous IFN therapy and clearance of HBeAg over the initial six months of treatment (HBeAg+ cohort only); and HBsAg levels (HBeAg- cohort only). It seems that both direct antiviral potential and host immune response are needed to achieve HBsAg clearance by NA therapy. Future studies are needed to validate these findings and to develop treatment regimens for HBsAg clearance in patients with chronic hepatitis B.

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**Conflict of interest** Dr. Kumada reports having received investigator, lecture, and consulting fees from Bristol-Myers Squibb, Dainippon Sumitomo Pharma Co., MSD K.K., and Toray Co. 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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# Effect of Type 2 Diabetes on Risk for Malignancies Includes Hepatocellular Carcinoma in Chronic Hepatitis C

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The aim of this retrospective cohort study was to assess the cumulative development incidence and predictive factors for malignancies after the termination of interferon (IFN) therapy in Japanese patients for hepatitis C virus (HCV). A total of 4,302 HCV-positive patients treated with IFN were enrolled. The mean observation period was 8.1 years. The primary outcome was the first onset of malignancies. Evaluation was performed using the Kaplan-Meier method and Cox proportional hazard analysis. A total of 606 patients developed malignancies: 393 developed hepatocellular carcinoma (HCC) and 213 developed malignancies other than HCC. The cumulative development rate of HCC was 4.3% at 5 years, 10.5% at 10 years, and 19.7% at 15 years. HCC occurred significantly ( $P < 0.05$ ) when the following characteristics were present: advanced histological staging, sustained virological response not achieved, male sex, advanced age of  $\geq 50$  years, total alcohol intake of  $\geq 200$  kg, and presence of type 2 diabetes (T2DM). T2DM caused a 1.73-fold enhancement in HCC development. In patients with T2DM, HCC decreased when patients had a mean hemoglobin A1c (HbA1c) level of  $< 7.0\%$  during follow-up (hazard ratio, 0.56; 95% confidence interval, 0.33-0.89;  $P = 0.015$ ). The cumulative development rate of malignancy other than HCC was 2.4% at 5 years, 5.1% at 10 years, and 9.8% at 15 years. Malignancies other than HCC occurred significantly when patients were of advanced age of  $\leq 50$  years, smoking index (package per day  $\times$  year) was  $\geq 20$ , and T2DM was present. T2DM caused a 1.70-fold enhancement in the development of malignancies other than HCC. **Conclusion:** T2DM causes an approximately 1.7-fold enhancement in the development of HCC and malignancies other than HCC in HCV-positive patients treated with IFN. In T2DM patients, maintaining a mean HbA1c level of  $< 7.0\%$  reduces the development of HCC. (HEPATOLOGY 2012;000:000-000)

**H**epatitis C virus (HCV) is one of the more common causes of chronic liver disease worldwide. Chronic hepatitis C is an insidiously progressive form of liver disease that relentlessly but silently progresses to cirrhosis in 20%-50% of cases over a period of 10-30 years.<sup>1,2</sup> In addition, HCV is a major risk factor for hepatocellular carcinoma (HCC).<sup>3-7</sup>

On the other hand, the prevalence of patients with type 2 diabetes mellitus (T2DM) is increasing in many nations, including Japan.<sup>8</sup> Thus, the

management of T2DM patients who are chronically infected with HCV is one of the most important issues confronted by physicians. Few studies have reported relationships between T2DM and total malignancies, including HCC in HCV patients. In addition, it is not clear whether the stringent control of T2DM is necessary for protecting the development of malignancies in HCV patients. This issue needs to be confirmed via long-term follow-up of a large cohort of patients at high risk of developing malignancy.

Abbreviations: CH, chronic hepatitis; CI, confidence interval; HbA1c, hemoglobin A1c; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HR, hazard ratio; IFN, interferon; LC, liver cirrhosis; SVR, sustained virological response; T2DM, type 2 diabetes mellitus; TAI, total alcohol intake.

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With this background in mind, the present study was initiated to investigate the cumulative incidence and risk factors of malignancies, including HCC after prolonged follow-up in HCV patients treated with interferon (IFN) monotherapy or combination therapy of IFN and ribavirin. The strengths of the current study are the large numbers of patients included and the long-term follow-up of patients.

## Patients and Methods

**Patients.** The number of patients who were diagnosed with chronic HCV infection and treated for the first time with IFN monotherapy or combination therapy between September 1990 and March 2009 in the Department of Hepatology, Toranomon Hospital, Tokyo, Japan, was 7,205. Of these, 4,302 patients met the following enrollment criteria: (1) no evidence of malignancies by physical examination, biochemical tests, abdominal ultrasonography, gastrofiberscope (or gastrography), or chest X-ray (or computed tomography); (2) features of chronic hepatitis or cirrhosis diagnosed via laparoscopy and/or liver biopsy within 1 year before the initiation of IFN therapy; (3) positivity for serum HCV-RNA before the initiation of IFN therapy; (4) period of  $\geq 1$  month to  $\leq 1$  year of IFN therapy; (5) negativity for hepatitis B surface antigens, antibody to hepatitis B core, or antimitochondrial antibodies in serum, as determined by radioimmunoassay, enzyme-linked immunosorbent assay, or indirect immunofluorescence assay; (6) age of  $\geq 30$  years to  $\leq 80$  years; (7) no underlying systemic disease, such as systemic lupus erythematosus or rheumatic arthritis; and (8) repeated annual examinations during follow-up. Annual examinations included biochemical tests, tumor marker (carcinoembryonic antigen, alpha-fetoprotein, and prostate-specific antigen [only in men]), and abdominal ultrasonography. Patients with were excluded from the study if they had illnesses that could seriously reduce their life expectancy or if they had a history of carcinogenesis.

The primary outcome was the first development of malignancy. The development of malignancies was diagnosed by clinical symptoms, tumor marker, imaging (ultrasonography, computed tomography, or magnetic resonance imaging), and/or histological

examination.<sup>9-15</sup> All of the studies were performed retrospectively by collecting and analyzing data from the patient records. The physicians in charge explained the purpose, method, and side effects of IFN therapy to each patient and/or the patient's family. In addition, the physicians in charge received permission for the use of serum stores and future use of stored serum. Informed consent for IFN therapy and future use of stored serum was obtained from all patients. The study was approved by the Institutional Review Board of our hospital.

**Medical Evaluation.** Body weight was measured in light clothing and without shoes to the nearest 0.1 kg. Height was measured to the nearest 0.1 cm. Height and weight were recorded at baseline, and body mass index was calculated as  $\text{kg}/\text{m}^2$ . All patients were interviewed by physicians or nurse staff in the Toranomon Hospital using a questionnaire that gathered information on demographic characteristics, medical history, and health-related habits, including questions on alcohol intake and smoking history.

The value for hemoglobin A<sub>1C</sub> (HbA<sub>1C</sub>) was estimated as a National Glycohemoglobin Standardization Program equivalent value (%). Patients were defined as having T2DM when they had a fasting plasma glucose level of  $\geq 126$  mg/dL and/or HbA<sub>1C</sub> level of  $\geq 6.5\%$ .<sup>16</sup>

Patients were regarded as hypertensive when systolic blood pressure was  $\geq 140$  mm Hg and/or diastolic blood pressure was  $\geq 90$  mm Hg for at least three visits. Smoking index (packs per day  $\times$  year) and total alcohol intake (TAI) were evaluated by the sum of before, during, and after the IFN therapy.

**Laboratory Investigation.** Diagnosis of HCV infection was based on detection of serum HCV antibody and positive RNA. Anti-HCV was detected using an enzyme-linked immunosorbent assay (ELISA II; Abbott Laboratories, North Chicago, IL). HCV genotype was examined via polymerase chain reaction assay, using a mixture of primers for the six subtypes known to exist in Japan, as reported.<sup>17</sup> HCV-RNA was determined using the COBAS TaqMan HCV test (Roche Diagnostics, Basel, Switzerland). The serum samples stored at  $-80^\circ\text{C}$  before IFN therapy were used. The linear dynamic range of the assay was 1.2-7.8 log IU/mL, and the undetectable samples were defined as negative. A sustained virological response (SVR) was

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**Table 1. Clinical Backgrounds at Initiation of Follow-up in Enrolled Patients**

| Variable   | Total             | HCC Group    | Non-HCC Malignancy Group | Without Events Group | P      |
|--|-------------------|--------------|--------------------------|----------------------|--------|
| No. of patients                                    | 4,302             | 393          | 213                      | 3,696                |        |
| Age, years   | 52.0 ± 11.8       | 55.8 ± 7.9   | 57.9 ± 9.1               | 51.3 ± 12.1          | <0.001 |
| Sex, male/female                                   | 2528/1774         | 272/121      | 129/84                   | 2127/1569            | <0.001 |
| Height, cm   | 163.0 ± 9.2       | 162.8 ± 8.3  | 163.3 ± 9.1              | 163.0 ± 9.3          | 0.772  |
| Weight, kg   | 61.4 ± 13.0       | 62.3 ± 10.6  | 60.8 ± 10.1              | 61.3 ± 13.4          | 0.142  |
| BMI  | 23.0 ± 4.0        | 23.4 ± 3.0   | 22.8 ± 2.8               | 23.0 ± 4.1           | 0.012  |
| Blood pressure, mm Hg                              |                   |              |                          |                      |        |
| Systolic   | 128 ± 18          | 132 ± 19     | 133 ± 20                 | 127 ± 17             | <0.001 |
| Diastolic  | 77 ± 13           | 80 ± 12      | 80 ± 13                  | 77 ± 13              | <0.001 |
| TAI, kg*   | 95 ± 92           | 151 ± 101    | 135 ± 81                 | 85 ± 89              | <0.001 |
| Smoking index*                                     | 6.4 ± 9.4         | 10.8 ± 11.1  | 12.5 ± 11.8              | 5.5 ± 8.7            | <0.001 |
| AST, IU/L  | 42 ± 44           | 64 ± 55      | 42 ± 31                  | 40 ± 42              | <0.001 |
| ALT, IU/L  | 44 ± 53           | 72 ± 63      | 43 ± 43                  | 42 ± 52              | <0.001 |
| GGT, IU/L  | 54 ± 61           | 63 ± 65      | 56 ± 45                  | 53 ± 38              | 0.007  |
| Albumin, g/dL                                      | 4.1 ± 0.3         | 4.1 ± 0.3    | 4.1 ± 0.2                | 4.1 ± 0.2            | 0.310  |
| Triglyceride, mg/dL                                | 101 ± 53          | 104 ± 54     | 105 ± 50                 | 100 ± 52             | 0.329  |
| Cholesterol, mg/dL                                 | 170 ± 32          | 165 ± 31     | 169 ± 33                 | 171 ± 32             | 0.025  |
| FPG, mg/dL   | 100 ± 22          | 110 ± 26     | 104 ± 22                 | 98 ± 21              | <0.001 |
| HbA1c, %, NSPG                                     | 5.6 ± 1.2         | 5.9 ± 1.4    | 5.7 ± 1.4                | 5.5 ± 1.1            | <0.001 |
| T2DM, +/-  | 267/4,035         | 63/330       | 34/179                   | 170/3,526            | <0.001 |
| Platelet count, × 10 <sup>4</sup> /mm <sup>3</sup> | 17.1 ± 5.1        | 13.7 ± 4.9   | 16.5 ± 5.4               | 17.5 ± 5.4           | <0.001 |
| Staging, LC/non-LC                                 | 433/3,869         | 113/285      | 27/189                   | 293/3,395            | <0.001 |
| HCV genotype, 1b/2a/2b/other                       | 2,721/995/458/128 | 283/52/20/38 | 121/62/18/12             | 2,317/881/420/78     | <0.001 |
| HCV RNA, log IU/mL                                 | 6.06 ± 1.05       | 6.22 ± 0.52  | 6.05 ± 0.86              | 6.04 ± 1.05          | 0.003  |
| IFN monotherapy†/combination therapy‡              | 2,861/1,441       | 358/35       | 175/38                   | 2,328/1,368          | <0.001 |
| Efficacy, SVR/non-SVR                              | 1,900/2,402       | 44/349       | 88/125                   | 1,768/1,928          | <0.001 |

Data are presented as no. of patients or mean ± SD.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; F, female; FPG, fasting plasma glucose; GGT, gamma-glutamyl transferase; HDL, high-density lipoprotein; M, male; NSPG, National Glycohemoglobin Standardization Program.

\*Smoking index is defined as packs per day × year. TAI and smoking index indicate the sum before and after first consultation.

†Outbreak of IFN monotherapy: recombinant IFN-α2a, n = 220, recombinant IFN-α2b, n = 183, natural IFN-α, n = 1,678, natural IFN-β, n = 691, total dose of IFN = 560 ± 164 megaunit. Outbreak of pegylated IFN monotherapy: pegylated IFN-α2a, n = 89, total dose of pegylated IFN = 7.52 ± 2.24 mg.

‡Outbreak of combination therapy: recombinant IFN-α2b + ribavirin, n = 335, total dose of IFN = 508 ± 184 megaunit, total dose of ribavirin = 160 ± 68 g; natural IFN-β + ribavirin, n = 101, total dose of IFN = 502 ± 176 megaunit, total dose of ribavirin = 156 ± 67 g; pegylated IFN-α2b + ribavirin, n = 1,005 cases, total dose of pegylated IFN = 4.14 ± 1.10 mg, total dose of ribavirin = 206 ± 58 g.

defined as clearance of HCV-RNA using the COBAS TaqMan HCV test 6 months after the cessation of IFN therapy.

**Evaluation of Liver Cirrhosis.** Status of liver was mainly determined on the basis of peritoneoscopy and/or liver biopsy. Liver biopsy specimens were obtained using a modified Vim Silverman needle with an internal diameter of 2 mm (Tohoku University style; Kakinuma Factory, Tokyo, Japan), fixed in 10% formalin, and stained with hematoxylin and eosin, Masson's trichrome, silver impregnation, and periodic acid-Schiff after diastase digestion. The size of specimens for examination was more than six portal areas.<sup>18</sup>

**Follow-up.** The observation starting point was 6 months after the termination of IFN therapy. After that, patients were followed up at least twice a year in our hospital. Physical examination and biochemical tests were conducted at each examination together with a regular checkup. In addition, annual examinations during follow-up were undertaken. When a

patient had complaints during follow-up, the physician in charge performed additional examinations based on symptoms. Four hundred eighteen patients were lost to follow-up. The final date of follow-up in 418 patients with loss of follow-up was regarded as the last consulting day. In addition, 881 patients were retreated with IFN. The final date of follow-up in 881 patients re-treated with IFN were regarded as the time of the initiation of IFN retreatment. Thus, 418 patients with loss of follow-up and 881 patients retreated with IFN were counted censored data in statistical analysis.<sup>19</sup> The mean follow-up period was 6.8 (SD 4.3) years in 418 patients with loss of follow-up and 7.5 (SD 4.8) years in 881 patients retreated with IFN. Censored patients were counted in the analysis.

**Statistical Analysis.** Clinical differences among three groups of patients with HCC with malignancies other than HCC without events were evaluated using the Kruskal-Wallis test. The cumulative development rates of malignancies were calculated using the Kaplan-Meier technique, and differences in the curves were

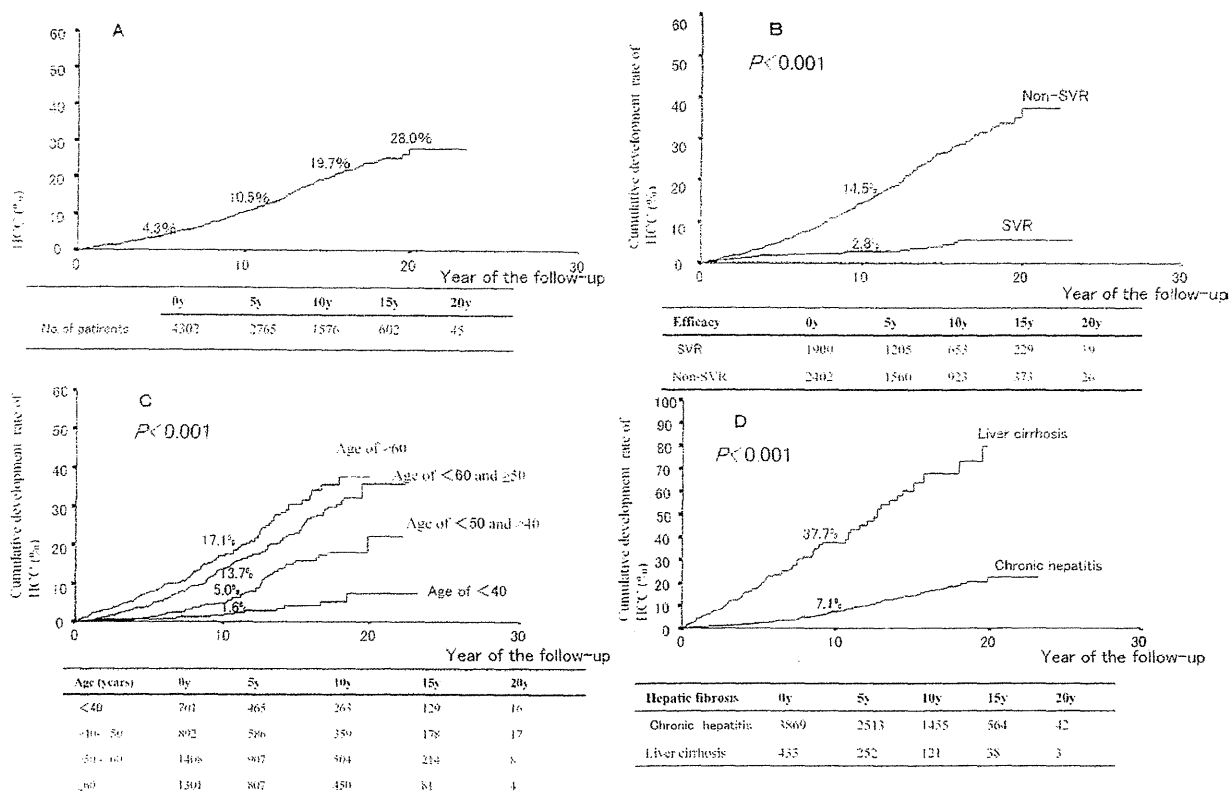


Fig. 1. Cumulative development rate of HCC (A) in total HCV patients treated with IFN therapy and based on the difference of (B) efficacy, (C) age, and (D) hepatic fibrosis.

tested using the log-rank test.<sup>20,21</sup> Independent risk factors associated with malignancies were studied using the stepwise Cox regression analysis.<sup>22</sup> The following variables were analyzed for potential covariates for incidence of primary outcome: (1) age, sex, T2DM, and hypertension at the initiation time of follow-up; (2) HCV genotype, HCV load, and hepatic fibrosis before IFN therapy; (3) average value of body mass index, aspartate aminotransferase, alanine aminotransferase, triglyceride, total cholesterol, and platelet count during follow-up; (4) sum value of smoking and alcohol before, during, and after the IFN therapy; and (5) efficacy of IFN therapy, combination of ribavirin, type of IFN, and total dose of IFN. A  $P < 0.05$  was considered statistically significant. Data analysis was performed using SPSS 11.5 for Windows (SPSS, Chicago, IL).

## Results

**Patient Characteristics.** Table 1 shows the baseline characteristics of the 4,302 enrolled patients at initiation of follow-up. The patients were divided into three groups: with HCC, with malignancies other than

HCC, and without events. There were significant differences in several baseline characteristics among the three groups. The SVR rate was 34.4% (985/2,861) in IFN monotherapy and 63.5% (915/1,441) in combination therapy of IFN and ribavirin. Thus, the number of patients with SVR was 1,900. The mean follow-up was 8.1 (SD 5.0) years.

**Development and Breakdown of Malignancies.** As shown in Table 1, 606 of 4,302 patients developed malignancies: 393 developed HCC and 213 developed malignancies other than HCC. HCC accounted for 33.3% (44/132) of malignancies in patients with SVR and 73.6% (349/474) in patients without SVR. The breakdown of malignancies other than HCC was as follows: stomach cancer,  $n = 36$ ; colon cancer,  $n = 35$ ; lung cancer,  $n = 20$ ; malignant lymphoma,  $n = 19$ ; pancreatic cancer,  $n = 12$ ; prostatic cancer,  $n = 16$ ; breast cancer,  $n = 15$ ; other cancers,  $n = 60$ .

**Predictive Factors for the Development of HCC.** The cumulative development rate of HCC was 4.3% at 5 years, 10.5% at 10 years, 19.7% at 15 years, and 28.0% at 20 years (Fig. 1A). The factors associated with the development of HCC are shown in Table 2. Multivariate Cox proportional hazards analysis



**Table 2. Predictive Factors for Development of HCC in Enrolled Patients**

| Variable  | Univariate Analysis |        | Cox Regression Analysis |        |
|---|---------------------|--------|-------------------------|--------|
|   | HR (95% CI)         | P      | HR (95% CI)             | P      |
| Age, years (per 10)   | 1.84 (1.64-2.06)    | <0.001 | 1.97 (1.71-2.28)        | <0.001 |
| Sex, male/female  | 1.47 (1.18-1.83)    | <0.001 | 1.67 (1.24-2.23)        | 0.001  |
| BMI, $\geq 22$ / $< 22$   | 1.37 (1.12-1.66)    | 0.002  |                         |        |
| T2DM, +/-   | 2.77 (2.13-3.60)    | <0.001 | 1.73 (1.30-2.30)        | <0.001 |
| Hypertension, +/-   | 1.32 (1.02-1.71)    | 0.036  |                         |        |
| Smoking index, $\geq 20$ / $< 20$ *                                 | 1.43 (1.14-1.79)    | 0.002  |                         |        |
| TAI, kg, $\geq 200$ / $< 200$ *                                     | 2.13 (1.74-2.61)    | <0.001 | 1.45 (1.11-1.88)        | 0.007  |
| AST, IU/L, $\geq 34$ / $< 34$                                       | 3.00 (2.40-3.89)    | <0.001 |                         |        |
| ALT, IU/L, $\geq 36$ / $< 36$                                       | 2.74 (2.16-3.42)    | <0.001 |                         |        |
| GGT, IU/L, $\geq 109$ / $< 109$                                     | 1.79 (1.19-2.46)    | 0.039  |                         |        |
| Albumin, g/dL, $< 3.9$ / $\geq 3.9$                                 | 1.92 (1.37-2.55)    | 0.015  |                         |        |
| Triglyceride, mg/dL, $\geq 100$ / $< 100$                           | 1.14 (0.94-1.37)    | 0.179  |                         |        |
| Cholesterol, mg/dL, $< 150$ / $\geq 150$                            | 1.38 (1.10-1.72)    | 0.004  |                         |        |
| Platelet count, $\times 10^4$ /mm <sup>3</sup> , $< 15$ / $\geq 15$ | 3.27 (2.56-4.17)    | <0.001 |                         |        |
| Histological diagnosis, LC/non-LC                                   | 7.09 (5.59-9.01)    | <0.001 | 5.01 (3.92-6.40)        | <0.001 |
| Combination of ribavirin, +/-                                       | 0.66 (0.45-0.97)    | 0.033  |                         |        |
| Type of IFN, $\alpha$ / $\beta$                                     | 1.10 (0.85-1.41)    | 0.474  |                         |        |
| Total dose of IFN, MU, $\geq 500$ / $< 500$                         | 1.12 (0.91-1.38)    | 0.291  |                         |        |
| HCV genotype, 1/2   | 1.67 (1.30-2.14)    | <0.001 |                         |        |
| HCV-RNA, log IU/mL, $\geq 5$ / $< 5$                                | 1.02 (0.98-1.05)    | 0.315  |                         |        |
| Efficacy, non-SVR/SVR   | 4.78 (3.47-6.59)    | <0.001 | 4.93 (3.53-6.89)        | <0.001 |

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; GGT, gamma-glutamyl transferase; HDL, high-density lipoprotein.

\*Smoking index is defined as packs per day  $\times$  year. TAI and smoking index indicate the sum before and after first consultation.

showed that HCC occurred when patients had liver cirrhosis (hazard ratio [HR], 5.01; 95% confidence interval [CI], 3.92-6.40;  $P < 0.001$ ), non-SVR (HR, 4.93; 95% CI, 3.53-6.89;  $P < 0.001$ ), age increments of 10 years (HR, 1.97; 95% CI, 1.71-2.28;  $P < 0.001$ ), T2DM (HR, 1.73; 95% CI, 1.30-2.30;  $P < 0.001$ ), male sex (HR, 1.67; 95% CI, 1.24-2.23;  $P = 0.001$ ), and TAI of  $\geq 200$  kg (HR, 1.45; 95% CI, 1.11-1.88;  $P = 0.007$ ). Fig. 1B-D and Fig. 2A-C show the cumulative development rates of HCC based on difference of IFN efficacy, age, hepatic fibrosis, TAI, sex, and T2DM. The 10-year cumulative rates of HCC after IFN therapy was determined to be 7.1% in 3,869 patients with chronic hepatitis and 37.7% in 433 patients with cirrhosis by using the Kaplan-Meier Method (Fig. 1D). Fig. 2D shows the development rates of HCC in T2DM patients according to difference of mean hemoglobin A1c (HbA1c) level during follow-up. HCC decreased when T2DM patients had a mean HbA1c level of  $< 7.0\%$  during follow-up (HR, 0.56; 95% CI, 0.33-0.89;  $P = 0.015$ ). The development of HCC was reduced by 44% in T2DM patients with a mean HbA1c level of  $< 7.0\%$  compared with those with a mean HbA1c level of  $\geq 7.0\%$ .

Table 3 shows the development rate of HCC and risk factors in four groups classified by the difference of hepatic fibrosis and efficacy of IFN therapy. The development rate of HCC per 1,000 person years was

1.55 in patients with chronic hepatitis (CH) at baseline and SVR (CH+SVR), 18.23 in patients with liver cirrhosis (LC) at baseline and SVR (LC+SVR), 13.53 in patients with chronic hepatitis at baseline and non-SVR (CH+non-SVR), and 50.43 in patients with LC at baseline and non-SVR (LC+non-SVR). The risk of HCC development in the CH+SVR group was advanced age, male sex, TAI of  $\geq 200$  kg, and T2DM. T2DM enhanced the development of HCC with statistical significance in three groups of CH+SVR, CH+non-SVR, and LC+non-SVR.

**Predictive Factors for Development of Malignancies Other than HCC.** The cumulative development rate of malignancies other than HCC was 2.4% at 5 years, 5.1% at 10 years, 9.8% at 15 years, and 18.0% at 20 years (Fig. 3A). The factors associated with the development of malignancies other than HCC are shown in Table 4. Malignancies other than HCC occurred when patients had age increments of 10 years (HR, 2.19; 95% CI, 1.84-2.62;  $P < 0.001$ ), smoking index of  $\geq 20$  (HR, 1.89; 95% CI, 1.41-2.53;  $P < 0.001$ ), and T2DM (HR, 1.70; 95% CI, 1.14-2.53;  $P = 0.008$ ). Fig. 3B-D shows the cumulative development rates of malignancies other than HCC based on difference of age, smoking index, and T2DM. Fig. 3E shows the risk of malignancies other than HCC in T2DM patients according to mean HbA1c level during follow-up. The HR of HCC development in

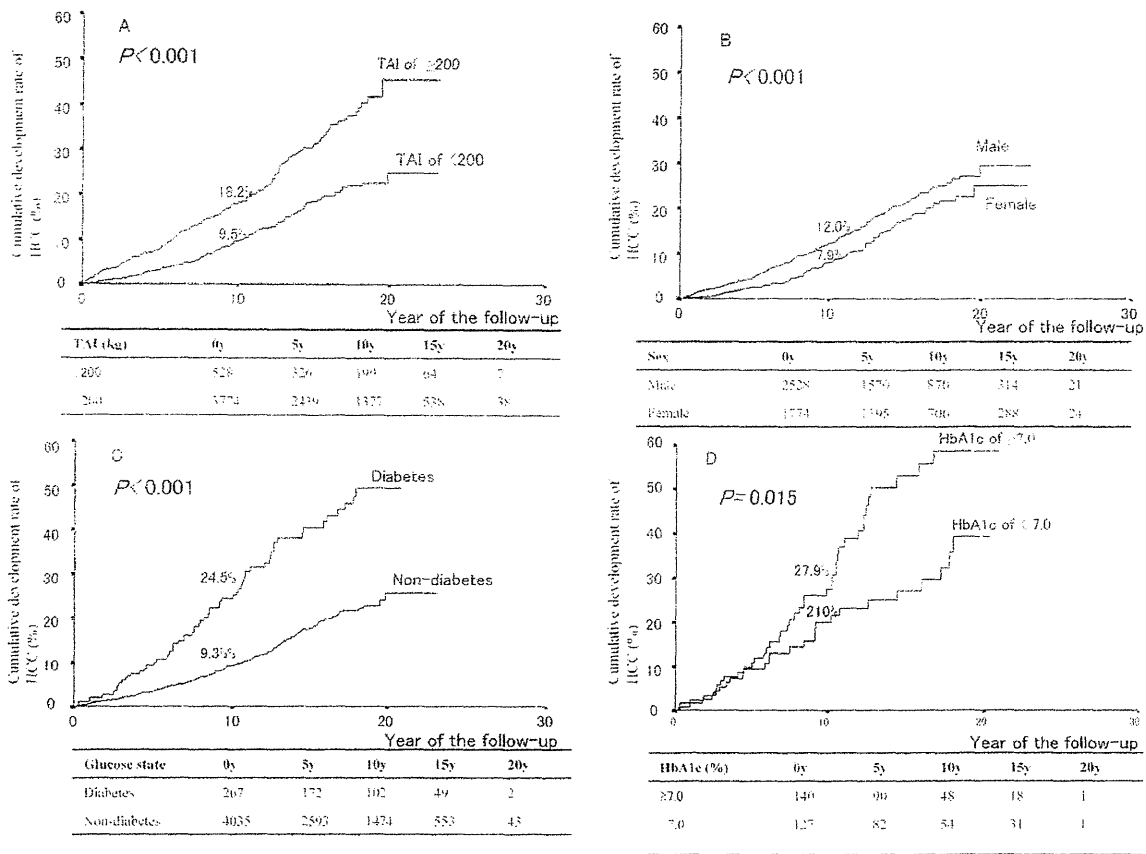


Fig. 2. Cumulative development rate of HCC based on the difference of (A) TAI, (B) sex, (C) diabetic state, and (D) mean HbA1c level during follow-up in T2DM patients.

patients with mean HbA1c level of <7.0% versus those with mean HbA1c level of  $\geq 7.0\%$  was 0.62 (95% CI, 0.31-1.23;  $P = 0.170$ ). There was no signif-

icant difference in development of malignancies other than HCC based on the difference of mean HbA1c level during follow-up. Table 5 shows the impact based

**Table 3. Development Rate of HCC Based on Hepatic Fibrosis and Efficacy of IFN Therapy**

| Variable                        | CH + SVR          | LC + SVR          | CH + Non-SVR     | LC + Non-SVR     |
|---------------------------------|-------------------|-------------------|------------------|------------------|
| No. of patients                 | 1,751             | 149               | 2,118            | 284              |
| Age, years                      | 51.7 $\pm$ 12.1   | 56.9 $\pm$ 9.8    | 51.5 $\pm$ 11.7  | 57.2 $\pm$ 9.9   |
| Sex, male/female                | 1,082/669         | 91/58             | 1,190/928        | 165/119          |
| HbA1c (% NSPG)                  | 5.5 $\pm$ 0.7     | 5.8 $\pm$ 0.8     | 5.7 $\pm$ 0.7    | 6.1 $\pm$ 0.8    |
| TAI, kg                         | 86 $\pm$ 91       | 104 $\pm$ 99      | 97 $\pm$ 90      | 129 $\pm$ 102    |
| Patients with T2DM              | 74                | 13                | 133              | 47               |
| Patients with HCC               | 22                | 22                | 233              | 116              |
| 1,000 person years of HCC       | 1.55              | 18.23             | 13.53            | 50.43            |
| Age, years (per 10)*            | 2.60 (1.48-4.58)  | 1.83 (0.95-3.55)  | 2.07 (1.75-2.46) | 1.09 (0.87-1.37) |
| P value                         | 0.001             | 0.070             | <0.001           | 0.477            |
| Sex, male/female*               | 3.42 (1.01-11.63) | 3.41 (1.00-11.63) | 1.34 (0.99-1.81) | 1.93 (1.25-3.00) |
| P value                         | 0.049             | 0.050             | 0.058            | 0.003            |
| TAI, kg, $\geq 200$ / $< 200$ * | 2.68 (1.14-6.34)  | 3.84 (1.83-9.85)  | 2.21 (1.65-2.95) | 1.54 (1.03-2.31) |
| P value                         | 0.024             | 0.004             | <0.001           | 0.038            |
| T2DM, +/-*                      | 4.76 (1.60-14.10) | 2.48 (0.57-10.86) | 2.53 (1.76-3.65) | 1.87 (1.16-3.01) |
| P value                         | 0.005             | 0.228             | <0.001           | 0.010            |

Abbreviations: CH + Non-SVR, patients with CH at baseline and non-SVR 6 months after IFN therapy; CH + SVR, patients with CH at baseline and SVR 6 months after IFN therapy; LC + Non-SVR, patients with LC at baseline and non-SVR 6 months after IFN therapy; LC + SVR, patients with LC at baseline and SVR 6 months after IFN therapy.

\*Hazard ratio (95% confidence interval) and P value by Cox proportional hazards analysis.

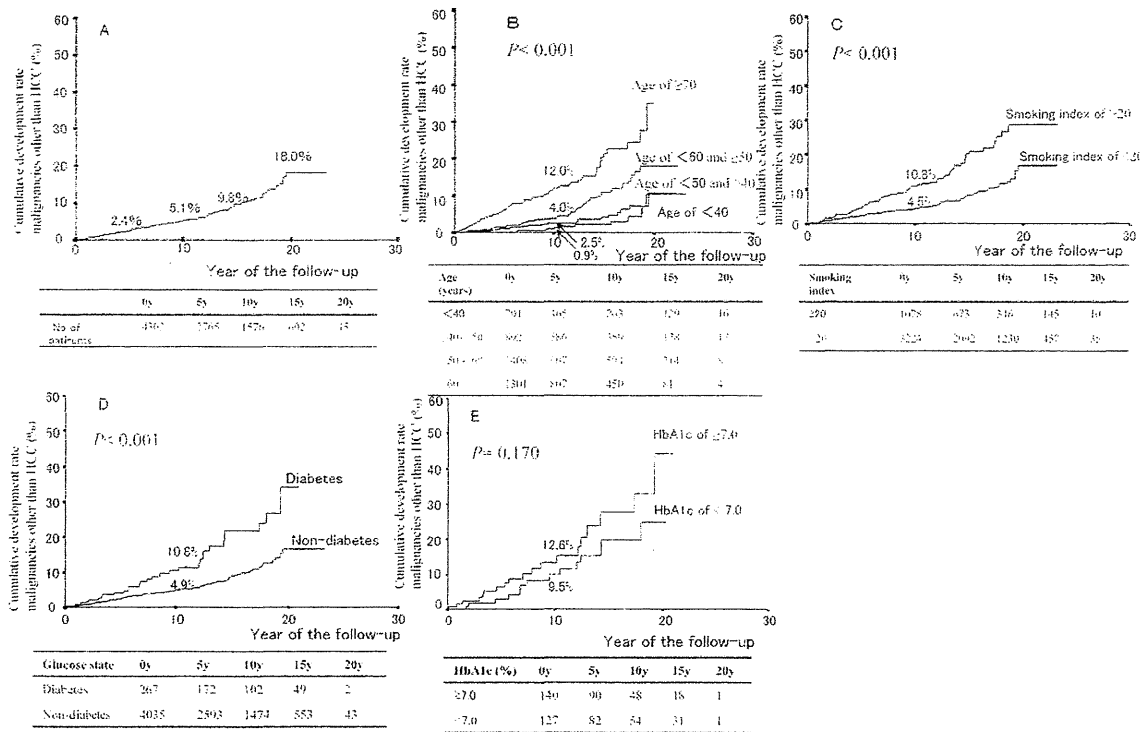


Fig. 3. Cumulative development rate of malignancies other than HCC (A) in total HCV patients treated with IFN therapy and based on the difference of (B) age, (C) smoking index, (D) diabetic state, and (E) mean HbA1c level during follow-up in T2DM patients.

on three factors of age, smoking index, and T2DM enhanced carcinogenesis of stomach, colon, lung, prostate, breast, and pancreas with statistical significance. HCC by using Cox regression analysis. Aging Smoking enhanced lung cancer and colorectal cancer

**Table 4. Predictive Factors for Development of Malignancies Other than HCC**

| Variables  | Univariate Analysis |         | Cox-Regression Analysis |        |
|--|---------------------|---------|-------------------------|--------|
|  | HR (95% CI)         | P       | HR (95% CI)             | P      |
| Age, years (per 10)  | 2.23 (1.88-2.65)    | < 0.001 | 2.19 (1.84-2.62)        | <0.001 |
| Sex, male/female   | 1.06 (0.79-1.40)    | 0.759   |                         |        |
| BMI, ≥22/<22   | 0.97 (0.75-1.24)    | 0.767   |                         |        |
| T2DM, 1/-  | 2.56 (1.76-3.72)    | <0.001  | 1.70 (1.14-2.53)        | 0.008  |
| Hypertension, +/-  | 2.33 (1.70-3.18)    | <0.001  |                         |        |
| Smoking index, ≥20/<20*                                      | 2.74 (2.06-3.65)    | <0.001  | 1.89 (1.41-2.53)        | <0.001 |
| TAI, kg, ≥200/<200*  | 1.77 (1.33-2.37)    | <0.001  |                         |        |
| AST, IU/L, ≥34/<34   | 0.89 (0.65-1.20)    | 0.412   |                         |        |
| ALT, IU/L, ≥36/<36   | 0.98 (0.72-1.34)    | 0.891   |                         |        |
| GGT, IU/L, ≥109/<109   | 1.26 (0.79-2.01)    | 0.350   |                         |        |
| Albumin, g/dL, <3.9/≥3.9                                     | 1.41 (0.90-2.04)    | 0.145   |                         |        |
| Triglyceride, mg/dL, ≥100/<100                               | 1.28 (1.03-1.60)    | 0.030   |                         |        |
| Total cholesterol, mg/dL, <150/≥150                          | 1.10 (0.82-1.46)    | 0.548   |                         |        |
| Platelet count, × 10 <sup>4</sup> /mm <sup>3</sup> , <15/≥15 | 1.39 (1.02-1.91)    | 0.038   |                         |        |
| Histological diagnosis, LC/non-LC                            | 1.77 (1.13-2.75)    | 0.012   |                         |        |
| Combination of ribavirin, +/-                                | 0.66 (0.44-0.97)    | 0.034   |                         |        |
| Type of IFN, α/β   | 1.05 (0.75-1.47)    | 0.789   |                         |        |
| Total dose of IFN, MU, ≥500/<500                             | 1.31 (0.96-1.77)    | 0.084   |                         |        |
| HCV genotype, 1/2  | 1.30 (0.80-2.93)    | 0.432   |                         |        |
| HCV RNA, log IU/mL, ≥5/<5                                    | 0.89 (0.50-1.23)    | 0.612   |                         |        |
| Efficacy, non-SVR/SVR  | 0.85 (0.64-1.12)    | 0.232   |                         |        |

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; GGT, gamma-glutamyl transferase.

\*Smoking index is defined as packs per day × year. TAI and smoking index indicate the sum before and after first consultation.

**Table 5. Impact Based on Age, Smoking Index, and Diabetes for Development of Malignancies Other than HCC**

| Malignancy                  | Age, Years (per 10) |        | Smoking Index, $\geq 20$ / $<20$ |       | Diabetes, +/-     |       |
|-----------------------------|---------------------|--------|----------------------------------|-------|-------------------|-------|
|                             | HR (95% CI)         | P      | HR (95% CI)                      | P     | HR (95% CI)       | P     |
| Gastric cancer (n = 36)     | 2.48 (1.62-3.78)    | <0.001 | 1.69 (0.83-3.43)                 | 0.146 | 2.29 (0.95-5.52)  | 0.065 |
| Colorectal cancer (n = 35)  | 1.91 (1.28-2.86)    | 0.002  | 2.27 (1.13-4.58)                 | 0.022 | 1.78 (0.68-4.66)  | 0.240 |
| Lung cancer (n = 20)        | 2.33 (1.35-4.01)    | 0.002  | 2.90 (1.25-6.74)                 | 0.013 | 1.53 (0.45-5.24)  | 0.496 |
| Prostatic cancer (n = 16)   | 2.84 (1.32-6.13)    | 0.008  | 1.89 (0.88-3.15)                 | 0.266 | 0.71 (0.09-5.47)  | 0.735 |
| Breast cancer (n = 15)      | 2.86 (1.30-6.29)    | 0.009  | 1.29 (0.17-10.19)                | 0.808 | 1.20 (0.16-9.39)  | 0.859 |
| Malignant lymphoma (n = 19) | 2.21 (1.26-3.88)    | 0.006  | 1.25 (0.44-3.56)                 | 0.671 | 1.39 (0.32-6.12)  | 0.663 |
| Pancreatic cancer (n = 12)  | 3.32 (1.44-7.65)    | 0.005  | 1.41 (0.45-4.82)                 | 0.578 | 3.75 (1.02-13.88) | 0.046 |

with statistical significance. In addition, T2DM enhanced the pancreatic cancer with statistical significance and tended to enhance the gastric cancer.

## Discussion

This study describes the development incidence of HCC or malignancies other than HCC after the termination of IFN therapy in HCV patients. Patients at Toranomon Hospital comprised mainly government employees, office workers, and business persons. Most patients were regularly recommended to undergo annual multiphasic health screening examinations. In the present study, patients who had undergone annual multiphasic health screening examinations were enrolled. The strengths of the present study are a prolonged follow-up in the large numbers of patients included.

The present study shows several findings with regard to the development incidence and predictive factors for total malignancies after IFN therapy for HCV patients. First, the 10-year cumulative rates of HCC after IFN therapy was determined to be 7.1% in 3,869 patients with chronic hepatitis and 37.7% in 433 patients with cirrhosis using the Kaplan-Meier method. Our previous studies showed via retrospective analysis that the 10-year cumulative rates of HCC were 12.4% for 456 patients with chronic hepatitis and 53.2% for 349 patients with cirrhosis.<sup>7,23</sup> Although patient selection bias for IFN treatment versus no treatment had been noted in the previous studies, the results suggest the possibility that IFN therapy reduces the development of HCC in HCV patients. Several historical data in Japan suggest that IFN therapy reduces the development of HCC in HCV patients.<sup>24-26</sup>

Second, HCC occurred with statistical significance when the following characteristics were present: non-SVR, advanced age, cirrhosis, TAI of  $\geq 200$  kg, male sex, and T2DM. T2DM caused a 1.73-fold enhancement in HCC development. Several authors have

reported an increased risk of HCC among patients with the following characteristics: non-SVR, cirrhosis, male sex, advanced age, and T2DM.<sup>24-28</sup> Our results show that physicians in charge of aged male patients with non-SVR, advanced fibrosis, TAI of  $\geq 200$  kg, and T2DM should pay attention to the development of HCC after IFN therapy. In addition, maintaining a mean HbA1c level of  $<7.0\%$  during follow-up reduced the development of HCC. This result indicates that stringent control of T2DM is important for protecting the development of HCC.

Third, the development rate of HCC per 1,000 person years was about 1.55 in 1,751 patients with chronic hepatitis at baseline and SVR. In these patients, the risk factors associated with HCC were advanced age, male sex, TAI, and T2DM. We compared the HCC development rate in patients with chronic hepatitis at baseline and SVR to the general population. A total of 5,253 individuals without HCV antibody and hepatitis B surface antigen, who underwent annual multiphasic health screening examinations in our hospital were evaluated as controls. Individuals with either of the following criteria were excluded: (1) illness that could seriously reduce their life expectancy or (2) history of carcinogenesis. They were selected by matching 3:1 with patients who had chronic hepatitis at baseline and SVR for age, sex, T2DM, and follow-up periods. In control individuals, the mean age was 51.7 years; the prevalence (number) of male patients was 61.8% (3,246); the prevalence (number) of T2DM patients was 4.2% (222); the mean follow-up period was 8.0 years. The number of development of HCC in control individuals was only five. This result suggests that the development rate of HCC in patients with chronic hepatitis at baseline and SVR is higher than that in the general population.

Fourth, HCC accounted for 33.3% in SVR patients and 73.6% in non-SVR patients. According to Matsuda et al.,<sup>29</sup> the outbreak of malignancies in the Japanese male population was observed in the following order in 2005: gastric cancer 20.4% > colon