Cumulative incidence and risk factors for the development of hepatocellular carcinoma in patients with chronic hepatitis B who achieved sustained disappearance of viremia by nucleos(t)ide analog treatment

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Aim: Nucleos(t)ide analog (NA) therapy has been reported to reduce the risk of hepatocellular carcinoma (HCC). However, some patients who achieve hepatitis B virus (HBV)-DNA disappearance from serum by NA develop HCC. In this study, we investigated the cumulative incidence and risk factors for HCC in patients with chronic hepatitis B (CHB) who achieved sustained disappearance of viremia by NA treatment.

Methods: A total of 133 CHB patients (median age, 51 years; 79 men [59%]; 28 with cirrhosis [21%]) who received NA therapy and achieved HBV-DNA disappearance from serum were analyzed retrospectively. We evaluated the cumulative incidence of HCC and risk factors associated with HCC based on data collected at the time of HBV-DNA disappearance.

Results: Thirteen patients developed HCC during the follow-up period. The 1-, 3-, and 5-year cumulative incidence of HCC was 0.0%, 7.8%, and 11.1%, respectively. In multivariate analysis, advanced age (hazard ratio [HR], 4.601; 95% confidence interval [CI], 1.220–17.351; P = 0.024), liver cirrhosis (HR, 5.563; 95% CI, 1.438–21.519; P = 0.013), and higher HBV core-related antigen (HBcrAg) levels (HR, 13.532; 95% CI, 1.683–108.815; P = 0.014) at the time of HBV-DNA disappearance were significantly associated with the development of HCC.

Conclusion: Our findings indicate the importance of continuous HCC surveillance especially in patients with advanced age, cirrhosis, and/or higher serum levels of HBcrAg, even if they achieve HBV-DNA disappearance.

Key words: aged, hepatitis B, hepatitis B virus core-related antigen, hepatocellular carcinoma, liver cirrhosis, nucleos(t)ide analog

INTRODUCTION

More than 350 million people worldwide are chronically infected with hepatitis B virus (HBV). Hepatitis B virus infection is one of the leading causes of cirrhosis and hepatocellular carcinoma (HCC); approximately 1 million patients die from HBV-related HCC and liver failure annually.

Patients with chronic hepatitis B (CHB) can be treated with nucleos(t)ide analogs (NAs), such as lamivudine (LAM), adefovir dipivoxil, entecavir (ETV), and tenofovir disoproxil fumarate. Nucleos(t)ide analogs suppress HBV replication through inhibition of HBV-DNA polymerase and are reported to reduce the risk of HCC incidence; however, such risk is not completely eliminated under NA treatment.

A large-scale study has shown that increased serum HBV-DNA load is an independent predictor of the development of HCC. In addition, decreased HBV-DNA load by NAs has been reported to reduce the risk of developing HCC or liver failure due to advanced fibrosis or cirrhosis. Some patients definitely develop HCC even if they can achieve sustained disappearance of viremia. Therefore, continuous surveillance of HCC is necessary even in such patients. However, the cumulative incidence and risk factors for the development of HCC in patients with CHB who achieved sustained disappearance of viremia on NA therapy is not well known.
In the present study, we analyzed the cumulative incidence of HCC and factors associated with the development of HCC in CHB patients who received NA and achieved sustained disappearance of viremia, and attempted to establish efficient surveillance of HCC in such patients.

METHODS

Hepatitis B virus markers

Serum HBV DNA levels were measured by the COBAS Amplicor HBV Monitor Test (Roche Diagnostics, Tokyo, Japan; detection range, 2.6–7.6 log copies/mL) before June 2008 and the COBAS TaqMan HBV Test (Roche Diagnostics; detection range, 2.1–9.0 log copies/mL) after June 2008. Hepatitis B virus DNA disappearance was defined as serum HBV DNA levels that were continuously under 2.1 log copies/mL by the COBAS TaqMan HBV Test. Serum HBV core-related antigen (HBcAg) levels were measured by a chemiluminescence enzyme immunoassay (Fujirebio, Tokyo, Japan; detection range, 3.0–7.0 log10 U/mL). Serum HB surface antigen (HBsAg) levels and HB envelope antigen (HBeAg) levels were measured by the Architect assay (Abbott Laboratories, Tokyo, Japan; detection range of HBsAg, 0.05–250 IU/mL). If the HBsAg level was >250 IU/mL, samples were diluted to 1:500 and re-examined.

Patient selection

Among 191 HBV-positive patients who had initiated NA therapy from 2000 to 2013, 179 patients achieved HBV-DNA disappearance from serum. The median duration of NA therapy was 6.8 years (range, 1–14.9 years). Patients were confirmed to be monoinfected with HBV and did not have other etiologies (e.g., alcohol, drug, autoimmune hepatitis, primary biliary cholangitis, or drug-induced liver disease). Additionally, patients did not receive interferon treatment prior to NA therapy. Among them, 33 patients who had developed HCC before the initiation of NA or before achievement of HBV-DNA disappearance from serum after introducing NA were excluded from the analysis. Four patients with incomplete clinical data were also excluded. Additionally, 9 patients who were determined to have achieved HBV-DNA disappearance by COBAS Amplicor test were excluded to unify the method. Thus, we finally analyzed 133 patients with CHB. Figure 1 shows the inclusion and exclusion criteria of this study.

The study protocol was approved by the Bioethics Committee of Nagoya University Hospital (Nagoya, Japan; approval no. 2016–0141). This study conformed to the ethical guidelines of the 1975 Declaration of Helsinki.
Statistical analysis

Continuous variables are expressed as medians (minimum–maximum) and categorical variables as frequencies. Continuous variables at the time of HBV-DNA disappearance were compared between HCC groups and non-HCC groups by the Mann–Whitney U-test. Categorical variables were compared by χ²-test or Fisher’s exact test. The cut-off levels were based on medians of each factor of each overall and subgroup analysis. The cumulative incidence of HCC was investigated by the Kaplan–Meier method and the differences between groups were analyzed using the log-rank test. Cox proportional hazards models were used to analyze factors associated with the development of HCC. Factors that showed differences at P-value <0.1 by univariate Cox regression analysis were evaluated by multivariate Cox regression analysis. Statistical analyses were carried out using IBM SPSS version 22 (IBM, Tokyo, Japan). A P-value <0.05 was considered statistically significant.

RESULTS

Patient characteristics and HCC incidence

Table 1 shows the clinical and virological characteristics of all patients at the time of HBV DNA disappearance from serum. The median (minimum–maximum) age was 51 years (range, 20–79 years) and 79 (59%) of patients were men. Twenty-eight (21%) patients had liver cirrhosis (LC). The median follow-up period after HBV-DNA disappearance was 4.8 years (range, 1.0–13.1 years) and 13 patients subsequently developed HCC (4 non-cirrhotic patients; 9 cirrhotic patients). The 1-, 3-, and 5-year cumulative incidence of

| Table 1 Characteristics of patients with chronic hepatitis B treated with nucleos(t)ide analogs at the time of hepatitis B virus DNA disappearance |
|---------------------------------|----------------|-----------------|-----------------|-----------------|
|                                | All (n = 133) | HCC (n = 13)    | Non-HCC (n = 120) | P-value         |
| Age, years                     | 51 (20–79)    | 57 (36–70)      | 50 (20–79)       | 0.039           |
| Sex, male / female             | 79/54         | 10/3            | 69/51            | 0.239           |
| Non-LC/LC                      | 105/28        | 4/9             | 101/19           | <0.001          |
| Treatment duration, years      | 0.6 (0.3–13.6)| 0.6 (0.3–6.0)   | 0.7 (0.3–13.6)   | 0.940           |
| AST, IU/L                      | 23 (13–66)    | 26 (16–53)      | 23 (13–66)       | 0.166           |
| ALT, IU/L                      | 23 (8–107)    | 27 (13–50)      | 23 (8–107)       | 0.124           |
| Serum bilirubin, mg/dl         | 0.8 (0.3–2.2) | 0.7 (0.5–1.6)   | 0.8 (0.3–2.2)    | 0.960           |
| GGT, IU/L                      | 27 (9–194)    | 44 (21–194)     | 25 (9–138)       | 0.002           |
| Serum albumin, g/dl            | 4.2 (2.5–5.2)| 4 (2.9–5)       | 4.2 (2.5–5.2)    | 0.201           |
| Platelet count, ×10³/μL        | 153 (30–420)  | 121 (30–171)    | 155 (31–420)     | 0.002           |
| HBeAg, + / −                   | 41/92         | 5/8             | 36/84            | 0.530           |
| HBcrAg, log10 U/mL             | 3.4 (<3.0–6.8)| 4.8 (<3.0–5.7)  | 3.4 (<3.0–6.8)   | 0.009           |
| HBsAg, IU/mL                   | 717.585 (0.00–21999.79) | 1616.72 (71.92–4352.98) | 669.13 (0.00–21999.79) | 0.254 |
| AFP, ng/mL                     | 4 (1–370)     | 6.5 (2–370)     | 4 (1–21)         | 0.023           |

Data are number of patients or median (range).
Continuous variables were compared by the Mann–Whitney U-test. Categorical variables were compared by the χ²-test or Fisher’s exact test.

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HCC was 0.0%, 7.8%, and 11.1%, respectively (Fig. 2). The period required for HBV-DNA disappearance after introduction of NAs was 0.6 (0.3–6.0) years and 0.7 (0.3–13.6) years in the HCC group and non-HCC group, respectively. It was not significantly different between the two groups ($P = 0.940$).

**Factors associated with incidence of HCC**

Predictive factors included in univariate analyses were age, sex, disease status (CH/LC), treatment duration, serum aspartate aminotransferase levels, serum alanine aminotransferase levels, serum bilirubin levels, serum γ-glutamyl transpeptidase levels, serum albumin levels, platelet counts, HBeAg positivity, serum HBcrAg levels, serum HBsAg levels and serum AFP levels. Multivariate Cox proportional hazards models showed that advanced age (hazard ratio [HR], 4.601; 95% confidence interval [CI], 1.220–17.351; $P = 0.024$), LC (HR, 5.563; 95% CI, 1.438–21.519; $P = 0.013$), and higher serum HBcrAg levels (HR, 13.532; 95% CI, 1.683–108.815; $P = 0.014$) were significantly associated with the development of HCC among the parameters examined at the time of HBV-DNA disappearance (Table 2). A stratified analysis of HCC incidence was carried out according to these risk factors, and the cumulative incidence of HCC at 1, 3, and 5 years was 0.0%, 10.3%, and 16.6% in patients ≥51 years of age and 0.0%, 5.2%, and 5.2% in patients <51 years of age, respectively ($P = 0.048$) (Fig. 3a). The cumulative incidence of HCC at 1, 3, and 5 years was 0.0%, 27.7%, and 32.2% in patients with cirrhosis compared with 0.0%, 2.2%, and 5.3% in patients without cirrhosis, respectively ($P < 0.001$) (Fig. 3b). The cumulative incidence of HCC at 1, 3, and 5 years was 0.0%, 13.6%, and 17.7% in patients with serum HBcrAg levels ≥3.4 log$_{10}$ U/mL and 0.0%, 0.0%, and 2.4% in patients with serum HBcrAg levels <3.4 log$_{10}$ U/mL respectively ($P = 0.005$) (Fig. 3c).

**Subanalysis for the incidence of HCC according to serum HBcrAg levels in patients with or without cirrhosis**

We also performed Cox regression analysis of factors associated with the development of HCC in patients with or without cirrhosis separately (Table 3). In these subgroup analyses, the median of serum HBcrAg levels in LC group was 4.0 log$_{10}$ U/mL, though the median in CH group remained 3.4 log$_{10}$ U/mL. Higher serum HBcrAg levels at the time of HBV DNA disappearance became a statistically significant factor for the development of HCC in the cirrhotic group in tendency ($P = 0.079$).

**Subanalysis for the incidence of HCC in patients according to HBeAg status**

We undertook subgroup analyses stratified according to HBeAg status. In these subgroup analyses, the median of serum HBcrAg levels in the HBeAg negative group was 3.0 log$_{10}$ U/mL, whereas the median in the HBeAg positive group was 5.6 log$_{10}$ U/mL. Multivariate Cox proportional hazards models showed that LC became a statistically significant factor associated with the development of HCC (HR, 7.646; 95% CI, 1.823–32.076; $P = 0.005$), in contrast, higher serum HBcrAg levels became a significant factor in tendency (HR, 7.254; 95% CI, 0.890–59.108; $P = 0.064$) in HBeAg negative patients (Table 4a). And in HBeAg positive patients, only LC became a statistically significant factor associated with the development of HCC (HR, 13.035; 95% CI, 1.448-117.350; $P = 0.022$) (Table 4b).

**Decline of serum HBcrAg levels from HBV-DNA disappearance to last follow-up date between HCC and non-HCC groups**

We also analyzed the decline of serum HBcrAg levels in both the HCC and non-HCC groups. Median serum HBcrAg levels at the time of last follow-up date were 4.5 ($< 3.0$–5.7) log$_{10}$ U/mL in the HCC group and 3.0 ($< 3.0$–6.8) log$_{10}$ U/mL in the non-HCC group ($P < 0.001$). The HCC group had significantly higher median serum HBcrAg levels not only at the time of HBV-DNA disappearance, but also at the time of last follow-up date compared with the non-HCC group. The decline of serum HBcrAg levels (the annual average decline in serum HBcrAg levels at the time between HBV-DNA disappearance and last follow-up date) was 0.04 (0.00–0.15) log$_{10}$ U/mL per year in the HCC group and 0.00 (0.00–0.90) log$_{10}$ U/mL per year in the non-HCC group ($P = 0.881$) (Fig. 4).

**DISCUSSION**

In the present study, we evaluated the cumulative incidence of HCC and risk factors for the development of HCC in patients with CHB who achieved sustained disappearance of viremia while on NA treatment. The cumulative incidence of HCC in these groups was higher than we expected. Advanced age, LC, and higher serum HBcrAg levels at the time of HBV-DNA disappearance were found to be independent risk factors for HCC.

Several reports have shown that advanced age is a risk factor of HCC incidence during NA treatment.\(^{15-17}\)
Table 2  Cox proportional hazards models of clinical and virological variables associated with the development of hepatocellular carcinoma in patients with chronic hepatitis B treated with nucleos(t)ide analogs

| Factor                       | Univariate analysis |                |                |                | Multivariate analysis |                |                |                |
|------------------------------|---------------------|----------------|----------------|----------------|-----------------------|----------------|----------------|----------------|
|                              | HR                  | 95% CI         | P-value        | HR             | 95% CI                | P-value        | HR             | 95% CI         |
| Age, years                   |                     |                |                |                |                       |                |                |                |
| <51 (n = 64)                 | 1.000               |                |                | 1.000          |                       |                |                |                |
| ≥51 (n = 69)                 | 3.395               | 0.934–12.340   | 0.063          | 4.601          | 1.220–17.351          | 0.024          |                |                |
| Sex                          |                     |                |                |                |                       |                |                |                |
| Female (n = 54)              | 1.000               |                |                | 1.000          |                       |                |                |                |
| Male (n = 79)                | 2.200               | 0.605–7.996    | 0.231          |                |                       |                |                |                |
| LC                           |                     |                |                |                |                       |                |                |                |
| – (n = 105)                  | 1.000               |                |                | 1.000          |                       |                |                |                |
| + (n = 28)                   | 9.552               | 2.938–31.054   | <0.001         | 5.563          | 1.438–21.519          | 0.013          |                |                |
| Treatment duration, years    |                     |                |                |                |                       |                |                |                |
| <0.6 (n = 59)                | 1.000               |                |                | 1.000          |                       |                |                |                |
| ≥0.6 (n = 74)                | 1.154               | 0.387–3.438    | 0.797          |                |                       |                |                |                |
| AST, IU/L                    |                     |                |                |                |                       |                |                |                |
| <23 (n = 56)                 | 1.000               |                |                | 1.000          |                       |                |                |                |
| ≥23 (n = 77)                 | 2.260               | 0.622–8.215    | 0.215          |                |                       |                |                |                |
| ALT, IU/L                    |                     |                |                |                |                       |                |                |                |
| <23 (n = 64)                 | 1.000               |                |                | 1.000          |                       |                |                |                |
| ≥23 (n = 69)                 | 1.914               | 0.589–6.216    | 0.280          |                |                       |                |                |                |
| Serum bilirubin, mg/dl       |                     |                |                |                |                       |                |                |                |
| <0.8 (n = 63)                | 1.000               |                |                | 1.000          |                       |                |                |                |
| ≥0.8 (n = 70)                | 0.734               | 0.247–2.186    | 0.579          |                |                       |                |                |                |
| GGT, IU/L                    |                     |                |                |                |                       |                |                |                |
| <27 (n = 66)                 | 1.000               |                |                | 1.000          |                       |                |                |                |
| ≥27 (n = 67)                 | 3.043               | 0.837–11.060   | 0.091          |                |                       |                |                |                |
| Serum albumin, g/dl          |                     |                |                |                |                       |                |                |                |
| <4.2 (n = 66)                | 1.000               |                |                | 1.000          |                       |                |                |                |
| ≥4.2 (n = 67)                | 0.749               | 0.244–2.294    | 0.612          |                |                       |                |                |                |
| Platelet count, /μL          |                     |                |                |                |                       |                |                |                |
| ≥153 × 10^3 (n = 67)         | 1.000               |                |                | 1.000          |                       |                |                |                |
| <153 × 10^3 (n = 66)         | 3.300               | 0.907–12.001   | 0.070          |                |                       |                |                |                |
| HBcAg – (n = 92)             | 1.000               |                |                | 1.000          |                       |                |                |                |
| + (n = 41)                   | 1.585               | 0.518–4.848    | 0.419          |                |                       |                |                |                |
| HBcrAg, log10 IU/ml          |                     |                |                |                |                       |                |                |                |
| <3.4 (n = 58)                | 1.000               |                |                | 1.000          |                       |                |                |                |
| ≥3.4 (n = 74)                | 10.474              | 1.359–80.710   | 0.024          | 13.532         | 1.683–108.815         | 0.014          |                |                |
| HBsAg, IU/mL                 |                     |                |                |                |                       |                |                |                |
| <717.585 (n = 61)            | 1.000               |                |                | 1.000          |                       |                |                |                |
| ≥717.585 (n = 61)            | 1.264               | 0.425–3.762    | 0.674          |                |                       |                |                |                |
| AFP, ng/mL                   |                     |                |                |                |                       |                |                |                |
| <4 (n = 56)                  | 1.000               |                |                | 1.000          |                       |                |                |                |
| ≥4 (n = 73)                  | 2.183               | 0.591–8.067    | 0.242          |                |                       |                |                |                |

Predictive factors are given at the time of hepatitis B virus DNA disappearance.

AFP, α-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CI, confidence interval; GGT, γ-glutamyl transpeptidase; HBcAg, hepatitis B core-related antigen; HBcrAg, hepatitis B core-related antigen; HBsAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma; HR, hazard ratio; LC, liver cirrhosis.

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Moreover, LC was reported to be a significant factor of HCC incidence during NA therapy.15–18 Hosaka et al. reported that age (HR, 1.06; 95% CI, 1.03–1.09) and cirrhosis (HR, 4.28; 95% CI, 1.88–9.73) were associated with HCC at the 5-year follow-up in propensity score-matched HBV patients with or without ETV treatment.15 Wang et al. also reported that advanced age (HR, 1.96; 95% CI, 1.29–2.99) and cirrhosis (HR, 21.32; 95% CI, 10.53–43.17) were independent risk factors for HCC in HBV patients with or without NA treatment.16 Moreover,

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**Figure 3** Comparison of cumulative incidence of hepatocellular carcinoma (HCC) in patients with chronic hepatitis B who achieved continuous hepatitis B virus (HBV)-DNA disappearance from serum following treatment with nucleos(t)ide analogs, according to factors at the time of HBV-DNA disappearance selected in multivariate analysis. (a) Cumulative incidence of HCC among patients ≥51 years of age (solid line) and patients <51 years of age (broken line). (b) Cumulative incidence of HCC among patients with liver cirrhosis (LC) (solid line) and patients without cirrhosis (broken line). (c) Cumulative incidence of HCC among patients with hepatitis B core-related antigen (HBcrAg) levels ≥3.4 log_{10} U/mL (solid line) and patients with HBcrAg levels <3.4 log_{10} U/mL (broken line).
Kurokawa et al. reported that age (HR, 3.20; 95% CI, 1.08–9.53) and cirrhosis (HR, 4.64; 95% CI, 1.75–12.4) during LAM treatment were significant risk factors for the development of HCC in HBV patients, and age was a significant risk factor for HCC development in chronic hepatitis patients treated with LAM.17 Orito et al. reported that LC status was a significant risk factor for the development of HCC during NA therapy.18 Our results were consistent

Table 3 Cox proportional hazards models of clinical and virological variables associated with the development of hepatocellular carcinoma in patients with chronic hepatitis B treated with nucleos(t)ide analogs, according to the disease status (chronic hepatitis [CH] or liver cirrhosis [LC])

| Factor                  | CH                                        | Univariate analysis | Multivariate analysis |
|-------------------------|-------------------------------------------|---------------------|-----------------------|
|                         | HR | 95% CI          | P-value | HR | 95% CI        | P-value |
| Age, years              |    |                 |         |    |                |         |
| <50 (n = 51)            | 1.00 |                |         | 1.00 |                |         |
| ≥50 (n = 54)            | 62.709 | 0.020–19567.698 | 0.313 | 2.006 | 0.209–19.290 | 0.546 |
| Sex                     |    |                 |         |    |                |         |
| Female (n = 45)         | 1.00 |                |         | 1.00 |                |         |
| Male (n = 60)           | 2.006 | 0.209–19.290 | 0.546 |
| Treatment duration, years |    |                 |         |    |                |         |
| <0.7 (n = 52)           | 1.00 |                |         | 1.00 |                |         |
| ≥0.7 (n = 53)           | 1.271 | 0.179–9.029 | 0.811 |
| AST, IU/L               |    |                 |         |    |                |         |
| <23 (n = 48)            | 1.00 |                |         | 1.00 |                |         |
| ≥23 (n = 57)            | 2.363 | 0.246–22.741 | 0.456 |
| ALT, IU/L               |    |                 |         |    |                |         |
| <23 (n = 52)            | 1.00 |                |         | 1.00 |                |         |
| ≥23 (n = 53)            | 2.804 | 0.292–26.965 | 0.372 |
| Serum bilirubin, mg/dL  |    |                 |         |    |                |         |
| <0.7 (n = 36)           | 1.00 |                |         | 1.00 |                |         |
| ≥0.7 (n = 69)           | 0.583 | 0.082–4.139 | 0.590 |
| GGT, IU/L               |    |                 |         |    |                |         |
| <27 (n = 58)            | 1.00 |                |         | 1.00 |                |         |
| ≥27 (n = 47)            | 3.386 | 0.352–32.570 | 0.291 |
| Serum albumin, g/dL     |    |                 |         |    |                |         |
| <4.2 (n = 50)           | 1.00 |                |         | 1.00 |                |         |
| ≥4.2 (n = 55)           | 0.407 | 0.042–3.934 | 0.437 |
| Platelet count, /μL     |    |                 |         |    |                |         |
| ≥159 × 10^3 (n = 63)    | 1.00 |                |         | 1.00 |                |         |
| <159 × 10^3 (n = 42)    | 1.299 | 0.182–9.255 | 0.794 |
| HBeAg                   |    |                 |         |    |                |         |
| – (n = 74)              | 1.00 |                |         | 0.939 | 0.098–9.029 | 0.956 |
| + (n = 31)              | 0.939 | 0.098–9.029 |         |
| HBcrAg, log_{10} U/mL   |    |                 |         |    |                |         |
| <3.4 (n = 49)           | 1.00 |                |         | 1.00 |                |         |
| ≥3.4 (n = 56)           | 3.051 | 0.315–29.497 | 0.335 |
| HBsAg, IU/mL            |    |                 |         |    |                |         |
| <766.04 (n = 47)        | 1.00 |                |         | 1.00 |                |         |
| ≥766.04 (n = 48)        | 1.078 | 0.152–7.660 | 0.940 |
| AFP, ng/mL              |    |                 |         |    |                |         |
| <4 (n = 49)             | 1.00 |                |         | 1.00 |                |         |
| ≥4 (n = 52)             | 1.769 | 0.160–19.516 | 0.641 |

(Continues)
with these reports and confirmed these results even in patients who achieved continuous HBV-DNA disappearance from serum. Our findings suggest that patients with advanced age or LC while on NA therapy should be carefully followed up as potential high-risk populations of HCC, even if they achieve HBV-DNA disappearance.

Serum HBcrAg concentration has been reported to correlate well with intrahepatic covalently closed circular DNA (cccDNA), which is considered the template of HBV replication.\textsuperscript{19–21} Covalently closed circular DNA comprises HBV core antigen, which is translated from pregenomic mRNA, HBeAg, and a 22-kDa precore protein (p22Cr)
antigen that is translated from precore mRNA.\textsuperscript{22,23} Hepatitis B virus DNA load decreases rapidly during NA therapy, however, cccDNA persists in the liver and HBcrAg levels can remain sustained for long periods.\textsuperscript{24,25} Persistent cccDNA is associated with various clinical events, including HCC. However, monitoring cccDNA concentrations is difficult in clinical practice because liver biopsy is necessary, and there is no consensus on a clinically approved

| Table 4 Cox proportional hazards models of clinical and virological variables associated with the development of hepatocellular carcinoma in patients with chronic hepatitis B treated with nucleos(t)ide analogs, according to serum hepatitis B envelope antigen (HBeAg) status |
|---------------------------------|----------------|----------------|----------------|-------------------------------|----------------|----------------|----------------|
| (a) HBeAg negative             |                |                |                |                               |                |                |                |
| Factor                         | HR             | 95% CI         | \(P\)-value   |                               | HR             | 95% CI         | \(P\)-value   |
| Age, years                     |                |                |                |                               |                |                |                |
| \(<52\ (n = 46)\)              | 1.000          |                |                |                               |                |                |                |
| \(\geq 52\ (n = 46)\)         | 73.596         | 0.246–22036.398| 0.140          |                               |                |                |                |
| Sex                            |                |                |                |                               |                |                |                |
| Female \((n = 38)\)            | 1.000          |                |                |                               |                |                |                |
| Male \((n = 54)\)              | 2.227          | 0.449–11.037   | 0.327          |                               |                |                |                |
| LC                             |                |                |                |                               |                |                |                |
| – \((n = 74)\)                 | 1.000          |                |                |                               | 1.000          |                |                |
| + \((n = 18)\)                 | 7.936          | 1.894–33.242   | 0.005          |                               | 7.646          | 1.823–32.076   | 0.005          |
| Treatment duration, years      |                |                |                |                               |                |                |                |
| \(<0.5\ (n = 39)\)            | 1.000          |                |                |                               |                |                |                |
| \(\geq 0.5\ (n = 53)\)        | 1.366          | 0.326–5.716    | 0.670          |                               |                |                |                |
| AST, IU/L                      |                |                |                |                               |                |                |                |
| \(<23\ (n = 43)\)             | 1.000          |                |                |                               |                |                |                |
| \(\geq 23\ (n = 49)\)         | 2.524          | 0.509–12.506   | 0.257          |                               |                |                |                |
| ALT, IU/L                      |                |                |                |                               |                |                |                |
| \(<23\ (n = 46)\)             | 1.000          |                |                |                               |                |                |                |
| \(\geq 23\ (n = 46)\)         | 1.517          | 0.363–6.352    | 0.568          |                               |                |                |                |
| Serum bilirubin, mg/dL         |                |                |                |                               |                |                |                |
| \(<0.8\ (n = 45)\)            | 1.000          |                |                |                               |                |                |                |
| \(\geq 0.8\ (n = 47)\)        | 0.565          | 0.135–2.364    | 0.434          |                               |                |                |                |
| GGT, IU/L                      |                |                |                |                               |                |                |                |
| \(<27\ (n = 47)\)             | 1.000          |                |                |                               |                |                |                |
| \(\geq 27\ (n = 45)\)         | 3.050          | 0.615–15.136   | 0.172          |                               |                |                |                |
| Serum albumin, g/dl            |                |                |                |                               |                |                |                |
| \(<4.2\ (n = 46)\)            | 1.000          |                |                |                               |                |                |                |
| \(\geq 4.2\ (n = 46)\)        | 0.712          | 0.169–2.988    | 0.642          |                               |                |                |                |
| Platelet count, /\mu L         |                |                |                |                               |                |                |                |
| \(\geq 152 \times 10^3\ (n = 46)\) | 1.000 |                |                |                               |                |                |                |
| \(<152 \times 10^3\ (n = 46)\) | 3.173 | 0.640–15.733 | 0.158 |                               |                |                |                |
| HBcrAg, log_{10} U/mL          |                |                |                |                               |                |                |                |
| \(<3.0\ (n = 47)\)            | 1.000          |                |                |                               | 1.000          |                |                |
| \(\geq 3.0\ (n = 45)\)        | 7.576          | 0.931–61.656   | 0.058          |                               | 7.254          | 0.890–59.108   | 0.064          |
| HBsAg, IU/mL                   |                |                |                |                               |                |                |                |
| \(<293.44\ (n = 42)\)         | 1.000          |                |                |                               |                |                |                |
| \(\geq 293.44\ (n = 43)\)     | 4.930          | 0.607–40.076   | 0.136          |                               |                |                |                |
| AFP, ng/mL                     |                |                |                |                               |                |                |                |
| \(<4\ (n = 39)\)              | 1.000          |                |                |                               |                |                |                |
| \(\geq 4\ (n = 50)\)          | 4.270          | 0.514–35.480   | 0.179          |                               |                |                |                |

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Conversely, an HBcrAg measuring system is simple, does not require liver biopsy, and can be readily measured in clinical practice. This system was also reported to have possible usefulness to predict necroinflammatory and fibrotic activity in liver tissues.\(^{27}\)

Several reports have shown a correlation between the development of HCC and HBcrAg during NA therapy. Kumada et al. reported that HBcrAg levels before treatment were independently associated with the development of HCC in propensity score-matched patients with or without NA therapy (HR, 2.77; 95% CI, 1.07–7.17).\(^{28}\)

Table 4 (Continued)

| Factor                        | Univariate analysis |           |           |          | Multivariate analysis |           |           |          |
|-------------------------------|---------------------|-----------|-----------|----------|-----------------------|-----------|-----------|----------|
|                               | HR                  | 95% CI    | P-value   | HR       | 95% CI                | P-value   |
| Age, years                    |                     |           |           |          |                       |           |
| <49 (n = 20)                  | 1.00                |           |           |          |                       |           |
| ≥49 (n = 21)                  | 0.670               | 0.112–4.026 | 0.662    |          |                       |           |
| Sex                           |                     |           |           |          |                       |           |
| Female (n = 16)               | 1.00                |           |           |          |                       |           |
| Male (n = 25)                 | 1.962               | 0.219–17.569 | 0.547   |          |                       |           |
| LC                            |                     |           |           |          |                       |           |
| − (n = 31)                    | 1.00                |           |           |          |                       |           |
| + (n = 10)                    | 13.035              | 1.448–117.35 | 0.022   |          |                       |           |
| Treatment duration, years     |                     |           |           |          |                       |           |
| <1.3 (n = 20)                 | 1.00                |           |           |          |                       |           |
| ≥1.3 (n = 21)                 | 1.950               | 0.325–11.711 | 0.465   |          |                       |           |
| AST, IU/L                     |                     |           |           |          |                       |           |
| <25 (n = 20)                  | 1.00                |           |           |          |                       |           |
| ≥25 (n = 21)                  | 3.311               | 0.370–29.624 | 0.284   |          |                       |           |
| ALT, IU/L                     |                     |           |           |          |                       |           |
| <25 (n = 20)                  | 1.00                |           |           |          |                       |           |
| ≥25 (n = 21)                  | 3.279               | 0.365–29.423 | 0.289   |          |                       |           |
| Serum bilirubin, mg/dL        |                     |           |           |          |                       |           |
| <0.8 (n = 18)                 | 1.00                |           |           |          |                       |           |
| ≥0.8 (n = 23)                 | 1.115               | 0.184–6.744 | 0.906   |          |                       |           |
| GGT, IU/L                     |                     |           |           |          |                       |           |
| <28 (n = 19)                  | 1.00                |           |           |          |                       |           |
| ≥28 (n = 22)                  | 2.477               | 0.276–22.240 | 0.418   |          |                       |           |
| Serum albumin, g/dL           |                     |           |           |          |                       |           |
| <4.2 (n = 20)                 | 1.00                |           |           |          |                       |           |
| ≥4.2 (n = 21)                 | 0.778               | 0.130–4.670 | 0.783   |          |                       |           |
| Platelet count, /μL           |                     |           |           |          |                       |           |
| ≥153 × 10\(^3\) (n = 21)     | 1.00                |           |           |          |                       |           |
| <153 × 10\(^3\) (n = 20)     | 3.510               | 0.391–31.467 | 0.262   |          |                       |           |
| HBcrAg, log\(_{10}\) U/mL     |                     |           |           |          |                       |           |
| <5.6 (n = 18)                 | 1.00                |           |           |          |                       |           |
| ≥5.6 (n = 22)                 | 1.986               | 0.306–12.910 | 0.473   |          |                       |           |
| HBsAg, IU/mL                  |                     |           |           |          |                       |           |
| <2222.77 (n = 18)             | 1.00                |           |           |          |                       |           |
| ≥2222.77 (n = 19)             | 2.068               | 0.332–12.877 | 0.436   |          |                       |           |
| AFP, ng/mL                    |                     |           |           |          |                       |           |
| <4 (n = 17)                   | 1.00                |           |           |          |                       |           |
| ≥4 (n = 23)                   | 1.112               | 0.185–6.674 | 0.908   |          |                       |           |

Predictive factors are given at the time of hepatitis B virus DNA disappearance.

AFP, α-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CI, confidence interval; GGT, γ-glutamyl transpeptidase; HBcrAg, hepatitis B core-related antigen; HBsAg, hepatitis B surface antigen; HR, hazard ratio; LC, liver cirrhosis.
Additionally, Honda et al. reported that HBcAg levels during NA therapy at the end of the follow-up period were significantly associated with the development of HCC (HR, 3.53; 95% CI, 1.52–9.63). Moreover, Hosaka et al. reported that HBcAg levels at the time of HCC diagnosis was a useful predictor of post-treatment recurrence of HCC during NA therapy (HR, 8.96; 95% CI, 1.94–41.4). Indeed, we also confirmed that HBcAg at the time of HCC development was significantly higher compared with that of non-HCC patients, however, the decline of serum HBcAg levels from the time of HBV-DNA disappearance from serum to the last follow-up date were not significantly different among HCC and non-HCC groups (Fig. 4). Therefore, in this study, we also showed that HBcAg not only at the time of HCC development but also at the time of HBV-DNA disappearance may be an useful marker to predict the development of HCC during NA therapy.

Recently Cheung et al. reported that a higher post-NA treatment HBcAg level was associated with an increased risk of HCC development in patients with undetectable serum HBV-DNA under NA therapy. They included chronic HBV carriers with undetectable serum HBV-DNA under NA therapy without HCC as control groups after matching for age, gender, baseline HBsAg status, cirrhosis status, serum HBV-DNA levels, and duration of NA therapy with HCC patients. However, in Cheung et al.’s study, the method for matching HCC and control groups is not well described, so there might be a selection bias and the data might be arbitrary. In contrast, we enrolled all patients in clinical practice. Also in that paper, the period after HBV-DNA disappearance was not evaluated, whereas in our study, it was included as an evaluating factor associated with the development of HCC.

There are several limitations associated with this study. First, it was a single-center and retrospective study. Second, we did not carry out liver biopsies in all patients. We primarily diagnosed cirrhosis based on imaging data. Finally, we could not analyze serum HBcAg levels at the start of NA therapy because this study was cross-sectional and retrospective. We should continue to investigate the validity of our findings with further follow-up of these patients as well as increasing the number of patients.

In conclusion, achieving HBV-DNA disappearance from serum does not completely eliminate the risk of HCC in CHB patients treated with NAs. It is important to continue HCC surveillance, especially in patients with advanced age, cirrhosis, and/or higher serum levels of HBcAg, even if such patients achieve HBV-DNA disappearance.

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