ORIGINAL RESEARCH

Discontinuation of nucleos(t)ide analogues is not associated with a higher risk of HBsAg seroreversion after antiviral-induced HBsAg seroclearance: a nationwide multicentre study

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ABSTRACT

Objective Direct comparison of the clinical outcomes between nucleos(t)ide analogue (NA) discontinuation versus NA continuation has not been performed in patients with chronic hepatitis B who achieved HBsAg-seroclearance. Whether NA discontinuation was as safe as NA continuation after NA-induced surface antigen of HBV (HBsAg) seroclearance was investigated in the present study.

Designs This multicentre study included 276 patients from 16 hospitals in Korea who achieved NA-induced HBsAg seroclearance: 131 (47.5%) discontinued NA treatment within 6 months after HBsAg seroclearance (NA discontinuation group) and 145 (52.5%) continued NA treatment (NA continuation group). Primary endpoint was HBsAg reversion and secondary endpoints included serum HBV DNA redetection and development of hepatocellular carcinoma (HCC).

Results During follow-up (median=26.9 months, IQR=12.2–49.2 months), 10 patients (3.6%) experienced HBsAg reversion, 6 (2.2%) showed HBV DNA redetection and 8 (2.9%) developed HCC. Compared with NA continuation, NA discontinuation was not associated with HBsAg reversion in both univariable (HR=0.45, 95% CI=0.12 to 1.76, log-rank p=0.24) and multivariable analyses (adjusted HR=0.65, 95% CI=0.16 to 2.59, p=0.54). The cumulative probabilities of HBsAg reversion at 1, 3 and 5 years were 0.8%, 2.3% and 5.0% in the NA discontinuation group, and 1.5%, 6.3% and 8.4% in the NA continuation group, respectively. NA discontinuation was not associated with higher risk of either HBV redetection (HR=0.83, 95% CI=0.16 to 4.16, log-rank p=0.82) or HCC development (HR=0.53, 95% CI=0.12 to 2.23, log-rank p=0.38).

Conclusion The discontinuation of NA was not associated with a higher risk of either HBsAg reversion, serum HBV DNA redetection or HCC development compared with NA continuation among patients who achieved HBsAg seroclearance with NA.

Significance of this study

What is already known on this subject?

- Surface antigen of HBV (HBsAg) seroclearance is regarded as a functional cure of chronic hepatitis B and is associated with favourable clinical outcomes.
- Whether discontinuation of nucleos(t)ide analogues (NAs) may result in worse clinical outcomes than continuous NA treatment has not been proven.

What are the new findings?

- NA discontinuation was not associated with a higher risk of either HBsAg reversion, serum HBV DNA redetection or hepatocellular carcinoma development compared with NA continuation among patients who achieved HBsAg seroclearance with NA.
- Cirrhosis and previous history of antiviral resistance might be associated with higher risks for HBsAg reversion and HBV DNA redetection, respectively.

How might it impact on clinical practice in the foreseeable future?

- Discontinuation of NAs could be recommended after HBsAg seroclearance.
- Patients with cirrhosis or previous history of antiviral resistance require more attention.

INTRODUCTION

Chronic hepatitis B (CHB) remains a global health problem and approximately 257 million people with regional variation are infected worldwide.[1] Due to the recent advances in oral antiviral agents, third-generation nucleos(t)ide analogues (NAs) enable a successful long-term virological response in patients with CHB.[2 3] Viral suppression with antiviral agents minimises HBV-induced hepatic necroinflammation

and consequently improves the prognosis of patients with CHB and decrease incidence of hepatocellular carcinoma (HCC) than those not treated.[2 4–9]

The seroclearance of surface antigen of HBV (HBsAg) is currently considered a functional cure of CHB and optimal endpoint of HBV treatment.[10–12] However, HBsAg seroclearance achieved through NA treatment is an infrequent clinical event and in a previous Korean study reported that annual HBsAg seroclearance rate was 0.33% during NA treatment.[13] NA-induced HBsAg seroclearance showed similar durability to spontaneous HBsAg seroclearance and was associated with favourable clinical outcome, if achieved.[13 14] However, clinicians hesitate to discontinue NAs even after HBsAg seroclearance because the rate of HBsAg seroreversion, which could worsen clinical outcome, was reported to be up to 4.8%–11.7% at 3 years after NA discontinuation.[13 14] Whether discontinuation rather than continuation of NAs may result in worse clinical outcomes has not been proven.[10–12]

In the present multicentre study, whether NA discontinuation in patients with CHB who have achieved HBsAg seroclearance with NA treatment is equally safe as NA continuation was determined. In addition, factors affecting HBsAg reversion, HBV DNA redetection and HCC development were evaluated.

MATERIALS AND METHODS

Patients

Data were collected between January 2008 and July 2018 from electronic medical records at 16 university-affiliated hospitals in Korea (online supplementary table 1). Patients were identified based on the following inclusion criteria: achieved HBsAg seroclearance during NA treatment for at least 6 months (NA induced); negativity for HBsAg confirmed at least twice with ≥6-month interval between confirmations. Exclusion criteria included <6 month of NA treatment duration, single HBsAg test without follow-up and <6 months of follow-up after HBsAg seroclearance (online supplementary methods). HBsAg seroclearance was defined as loss of HBsAg accompanied by undetectable serum HBV DNA two or more times with ≥6-month interval to minimise the chance of false-negative HBsAg, and the first date of both negativity for HBsAg and undetectable serum HBV DNA was set as the index date. Cirrhosis was clinically identified using imaging and laboratory tests (online supplementary methods). The study subjects were classified into two groups based on maintenance of NAs after HBsAg seroclearance: patients who discontinued NA treatment within 6 months after the index date (NA discontinuation group) or patients who maintained NA treatment for ≥6 months after the index date (NA continuation group).

Outcomes and assessment

Primary outcome was HBsAg reversion. HBsAg test was performed every 3–6 months after HBsAg seroclearance, and time-to-HBsAg reversion was measured from the time of HBsAg seroclearance to the time when HBsAg reversion was detected. Secondary outcomes included HBV DNA redetection and HCC development. A composite endpoint (HBsAg redetection and/or HBV DNA) was used as a post hoc secondary endpoint. Quantification test of serum HBV DNA was performed every 3–6 months. Time-to-redetection of HBV DNA was measured from the time when HBV DNA was not detected to the time when HBV DNA was detected. HCC was diagnosed according to the American Association for the Study of Liver Disease (AASLD) guidelines (online supplementary methods).[15] Patients in the NA continuation group were censored at the point of NA discontinuation if they stopped NA treatment ≥6 months after HBsAg seroclearance.

Routine laboratory tests were performed at baseline and each follow-up using automated techniques (online supplementary table 1). Serum HBsAg was qualitatively measured using tests with very high sensitivity (lower limit of detection <0.02 IU/mL) and specificity (>99.5%) (online supplementary methods). HCC surveillance was performed using ultrasound, dynamic CT or dynamic MRI every 4–6 months in both groups.

Statistical analysis

Continuous variables were denoted by means±SD and compared using two-sample t test. Categorical variables were compared using χ^2 test. Non-parametric variables were compared using Mann-Whitney U test. Kaplan-Meier curves were generated for time to respective endpoints, and the log-rank test was used for group comparisons. When events did not occur in one group, the Firth regression method was used for group comparison.[16] Unadjusted HRs were estimated using the Cox proportional hazards model. Univariable and multivariable Cox proportional hazard analyses were performed to assess the effects of baseline characteristics on each outcome of interest. Variables with $p<0.20$ in univariable analyses as well as a variable of interest (ie, NA discontinuation) were used in multivariable analyses. All statistical tests were two sided and statistical significance was set at $p<0.05$. All statistical analyses were performed using Stata/SE 14.0 (StataCorp, College Station, Texas, USA).

RESULTS

Baseline characteristics

A total of 276 patients with CHB who achieved HBsAg seroclearance during NA treatment were included in this study: 131 patients (47.5%) were included in the NA discontinuation group and the remaining 145 patients (52.5%) in the NA continuation group (figure 1). Median duration of NA treatment before HBsAg seroclearance was 74.7 months (IQR, 37.9–122.2 months). The presence of cirrhosis was more common in the NA continuation group (49.0% vs 29.8%, p=0.001; table 1). Among patients with cirrhosis, 92.6% were diagnosed based on radiological findings and the remaining patients were clinically diagnosed with cirrhosis based on the presence of oesophageal varices with a risk of chronic liver disease. Median serum ALT levels were significantly different between the two groups (20 IU/L vs 18 IU/L, p=0.002). The positivity of anti-HBs was more frequent in the NA continuation group than the NA discontinuation group (p<0.001). Among the antiviral agents, lamivudine was more commonly used in the NA discontinuation group (p<0.001), while both entecavir and tenofovir disoproxil fumarate were more commonly used in the NA continuation group (both p<0.05).

HBsAg reversion

Median follow-up duration after HBsAg seroclearance was 26.9 months (IQR, 12.2–49.2 months): 38.4 months in the NA discontinuation group and 17.1 months in the NA continuation group. During the follow-up period, a total of 10 patients (three in the NA discontinuation group and seven in the NA continuation group) experienced HBsAg reversion without HBV DNA redetection. The overall HBsAg reversion risk was 1.15 cases per 100 person-years (0.71 cases per 100 person-years in the NA discontinuation group and 1.57 per 100 person-years in the NA continuation group). Among 10 cases of HBsAg reversion, eight

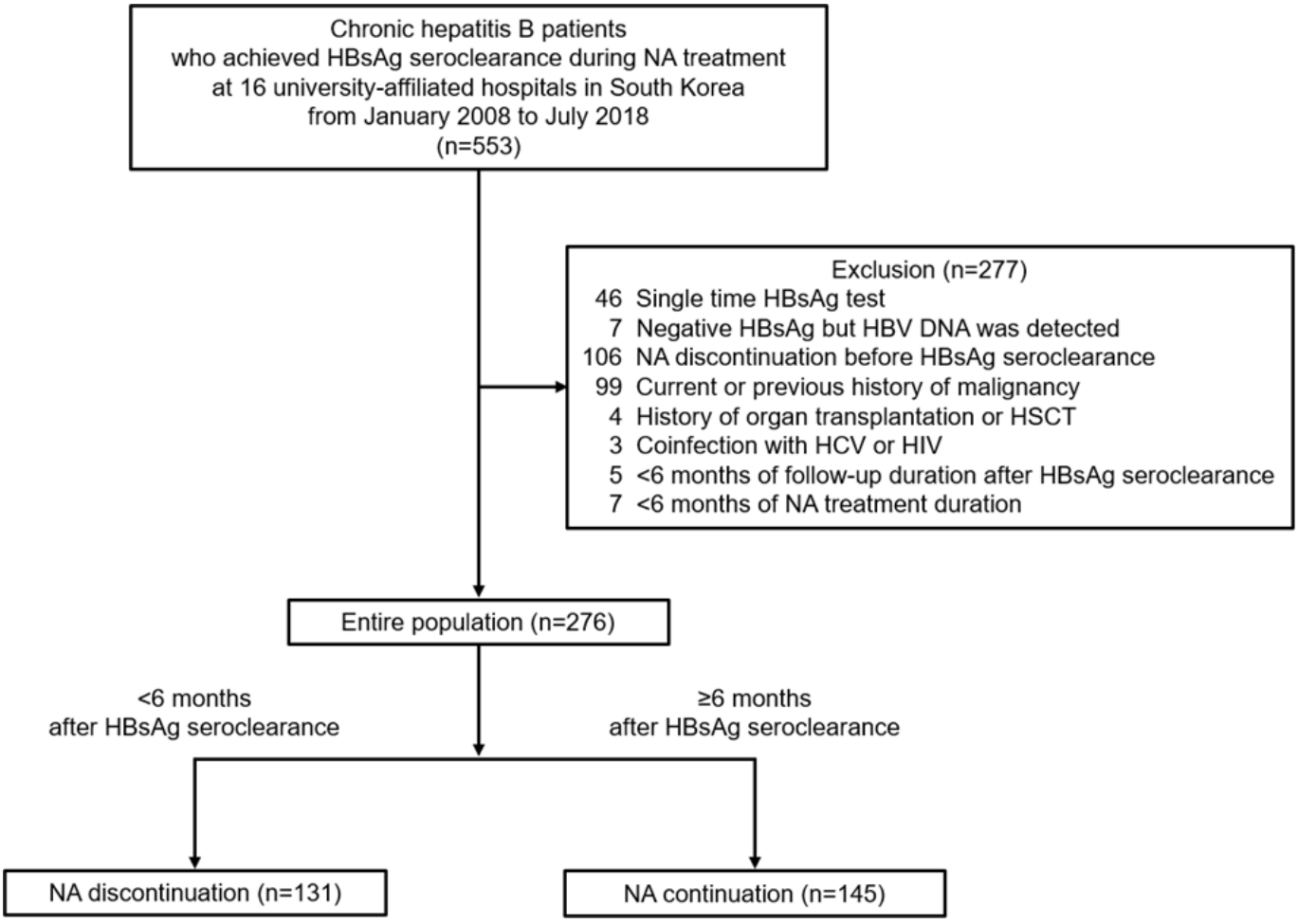

Figure 1 CONSORT diagram of the study. Of the 553 eligible patients, 277 were excluded from study according to inclusion and exclusion criteria. Finally, 276 patients were included in this study: 131 patients in the NA discontinuation group and 145 patients in the NA continuation group. CHB, chronic hepatitis B; CONSORT, Consolidated Standards of Reporting Trials; HBsAg, surface antigen of HBV; HSCT, haematopoietic stem cell transplantation; NA, nucleos(t)ide analogue.

showed transient HBsAg reversion and became negative again within 6 months of the next follow-up. However, two patients were persistently positive for HBsAg after HBsAg reversion and both patients were in the NA continuation group. For the entire study population, time-to-HBsAg reversion in the NA discontinuation group was not significantly different compared with the NA continuation group (HR=0.45, 95% CI=0.12 to 1.76, log-rank p=0.24; figure 2A). The cumulative rates of HBsAg reversion at 1, 3 and 5 years were 0.8%, 2.3% and 5.0% in the NA discontinuation group and 1.5%, 6.3% and 8.4% in the NA continuation group, respectively.

The presence of cirrhosis at the index date was significantly associated with a higher risk of HBsAg reversion (HR=4.98, 95% CI=1.06 to 23.51, log-rank p=0.04; online supplementary figure 1A), but not at the beginning of NA treatment (HR=1.55, 95% CI=0.44 to 5.41, p=0.49; online supplementary figure 1B).

Based on multivariable analysis (table 2), NA discontinuation was not an independent risk factor for HBsAg reversion (adjusted HR (aHR)=0.65, 95% CI=0.16 to 2.59, p=0.54) after adjusting for the presence of cirrhosis. Fortunately, no subjects who experienced HBsAg reversion experienced HBV DNA redetection or hepatitis flare-up.

Sensitivity analyses after excluding patients who had a history of two or more types of NA use (n=198), which suggests a history of previous NA-resistant mutation, showed no significant difference in HBsAg reversion between the NA continuation and NA discontinuation groups (HR=1.14, 95% CI=0.23 to 5.64, log-rank p=0.87; figure 2B). NA discontinuation was not associated with HBsAg reversion risk in subgroups of both cirrhotic patients (n=110, HR=0.62, 95% CI=0.12 to 3.10, log-rank p=0.56; figure 2C) and non-cirrhotic patients (n=166, HR=0.61, 95% CI=0.04 to 9.98, log-rank p=0.72; figure 2D). In the NA discontinuation group, because only one event of HBsAg reversion was observed, evaluation of the effects of anti-HBs on HBsAg reversion was limited.

When the patients were recategorized at a cut-off of 12 months after HBsAg seroclearance, HBsAg reversion was slightly more frequent in the NA continuation ≥12 months group than in the NA discontinuation <12 months group although statistical significance was not reached (HR=0.26, 95% CI=0.07 to 1.01, p=0.052; online supplementary figure 2A). HBV DNA redetection rate also did not differ between the two groups (HR=7.68, 95% CI=0.91 to 1002.23, p=0.06; online supplementary figure 2B). Collectively, the results indicate that longer consolidation treatment may have no beneficial effect in preventing HBsAg seroreversion or HBV DNA redetection. When the patients were divided based on 10 mIU/mL and 100 mIU/mL cut-off values of anti-HBs titre at the index date, anti-HBs titre was not associated with HBsAg reversion (p=0.052 and 0.44; online supplementary

Table 1 Baseline characteristics at the time of HBsAg seroclearance

	Total (n=276)	NA discontinuation (n=131)	NA continuation (n=145)	p value
Age (y), mean±SD	53.7±9.8	52.8±11.3	54.6±8.2	0.15
Male, n (%)	174 (63.0%)	84 (64.1%)	90 (62.1%)	0.72
Cirrhosis, n (%)	110 (39.9%)	39 (29.8%)	71 (49.0%)	0.001
Platelet (10^9/L), mean±SD	170.9±61.6	175.3±59.8	167.1±63.1	0.27
ALT (IU/L), median (IQR)	19 (14–28)	18 (13–25)	20 (14–33)	0.002
Prothrombin time (INR), mean±SD	1.02±0.10	1.02±0.09	1.02±0.11	0.5
Child-Pugh class, n (%)				0.18
A	95 (96.9%)	36 (100%)	59 (95.2%)	
B	1 (1.0%)	0 (0%)	1 (1.6%)	
C	2 (2.0%)	0 (0%)	2 (3.2%)	
FIB-4 score, n (%)				0.76
<1.45	115 (42.4%)	55 (43.3%)	60 (41.7%)	
1.45–3.24	127 (46.9%)	59 (46.5%)	69 (47.2%)	
≥3.25	29 (10.7%)	13 (10.2%)	18 (11.1%)	
APRI, n (%)				0.24
<0.5	212 (78.2%)	103 (81.1%)	109 (75.7%)	
0.5–1.4	50 (18.4%)	22 (17.3%)	28 (19.4%)	
≥1.5	9 (3.3%)	2 (1.6%)	7 (4.9%)	
NA treatment duration (months), median (IQR)	74.7 (37.9–122.2)	82.8 (46.2–122.5)	62.6 (27.7–119.1)	0.06
Positive anti-HBs, n (%)*	99 (36.7%)	34 (26.2%)	65 (46.4%)	<0.001
Positive anti-HBs at the time of NA discontinuation, n (%)	105 (38.0%)	35 (41.2%)	N/A	N/A
Antiviral agent, n (%)				
Lamivudine	122 (44.2%)	75 (57.2%)	47 (32.4%)	<0.001
Adefovir	48 (17.4%)	26 (19.8%)	22 (15.2%)	0.31
Telbivudine	12 (4.4%)	7 (5.3%)	5 (3.4%)	0.44
Clevudine	14 (5.1%)	8 (6.1%)	6 (4.1%)	0.46
Entecavir	115 (41.7%)	39 (29.8%)	76 (52.4%)	<0.001
Tenofovir DF	52 (18.8%)	16 (12.2%)	36 (24.8%)	0.008
Exposure to two or more types of NAs	79 (28.6%)	37 (28.2%)	42 (29.0%)	0.89

Data were expressed as mean±SD, median (IQR) or n (%).
*Six patients lacking anti-HBs at baseline were excluded.
ALT, alanine aminotransferase; anti-HBs, hepatitis B surface antibody; APRI, aspartate aminotransferase to platelet ratio index; DF, disoproxil fumarate; FIB-4, Fibrosis-4; HBsAg, surface antigen of HBV; INR, international normalised ratio; N/A, not applicable; NA, nucleos(t)ide analogue.

figure 3A,B) or HBV DNA redetection (p=0.46 and 0.09; online supplementary figure 3C,D).

HBV DNA redetection

Six patients (three in the NA discontinuation group and three in the NA continuation group) had serum HBV DNA redetected after HBsAg seroclearance during the follow-up period; however, none showed HBsAg reversion. The median level of HBV DNA was 225 IU/mL (range, 31–1850 IU/mL) at the time of HBV DNA redetection. Significant serum ALT elevation was not observed at the time of HBV DNA redetection, and all six patients showed transient HBV DNA redetection. Time-to-HBV DNA redetection was not significantly different between the NA continuation and NA discontinuation groups (HR=0.83, 95% CI=0.16 to 4.16, log-rank p=0.82; figure 3A). Based on multivariable analysis (table 3), NA discontinuation was not an independent risk factor for HBV DNA redetection (aHR=0.66, 95% CI=0.12 to 3.50, p=0.62) after adjusting for exposure to two or more types of NAs.

Among 197 patients after excluding patients who had a history of two or more types of NA use, only one patient in the NA continuation group showed HBV DNA redetection and significant difference was not observed between the two groups. HBV DNA redetection was not observed in any patient with cirrhosis. In the non-cirrhotic subgroup, NA discontinuation was not associated with HBV DNA redetection risk (HR=0.47, 95% CI=0.09 to 2.48, log-rank p=0.37; online supplementary figure 4). In the NA discontinuation group, significant difference in HBV DNA redetection based on positivity of anti-HBs at the time of NA discontinuation was not observed between the two groups (HR=3.66, 95% CI=0.33 to 40.62, log-rank p=0.26; online supplementary figure 5).

Regarding a composite endpoint (HBsAg reversion and/or HBV DNA redetection), significant difference was not observed between the NA continuation and NA discontinuation groups among the entire study population based on both univariable (HR=0.65, 95% CI=0.24 to 1.79, log-rank p=0.40; figure 3B) and multivariable analyses (aHR=0.64, 95% CI=0.22 to 1.65, p=0.32; table 4). None of the pretreatment variables showed significant association with HBsAg reversion and/or HBV DNA redetection (see online supplementary tables 2–4).

HCC development

During the follow-up period, eight patients (three in the NA discontinuation group and five in the NA continuation group) were diagnosed with HCC after HBsAg seroclearance, and the range of duration from the index date to HCC development was 24.5–70.3 months. The risk of developing HCC was not significantly different between the two groups based on both univariable (HR=0.53, 95% CI=0.12 to 2.23, log-rank p=0.38; figure 4A) and multivariable analyses (aHR=1.36, 95% CI=0.22 to 8.20, p=0.74; online supplementary table 5). In a subgroup of 110 patients with cirrhosis, the frequency of HCC development was not different between the NA discontinuation group and the NA continuation group (HR=0.93, 95% CI=0.17 to 5.11, log-rank p=0.94; figure 4B). The result was maintained even after excluding Child-Pugh class B or C patients, who are supposedly at higher risk of HCC (HR=0.63, 95% CI 0.14 to 2.84, log-rank p=0.54). NA discontinuation was not associated with HCC development in the 198 patients who did not use two or more types of NAs (HR=0.47, 95% CI=0.08 to 2.61, log-rank p=0.38; figure 4C).

DISCUSSION

In the present multicentre study including 276 patients with CHB who achieved NA-induced HBsAg seroclearance, results showed that patients who discontinued NAs within 6 months after HBsAg seroclearance had no higher risk of HBsAg reversion, HBV DNA redetection or HCC development than patients who maintained NAs ≥6 months after HBsAg seroclearance. Although recent guidelines from the European Association for the Study of the Liver recommend that NAs should be discontinued after confirmed HBsAg loss regardless of anti-HBs,[11] whether NA discontinuation may result in different or similar

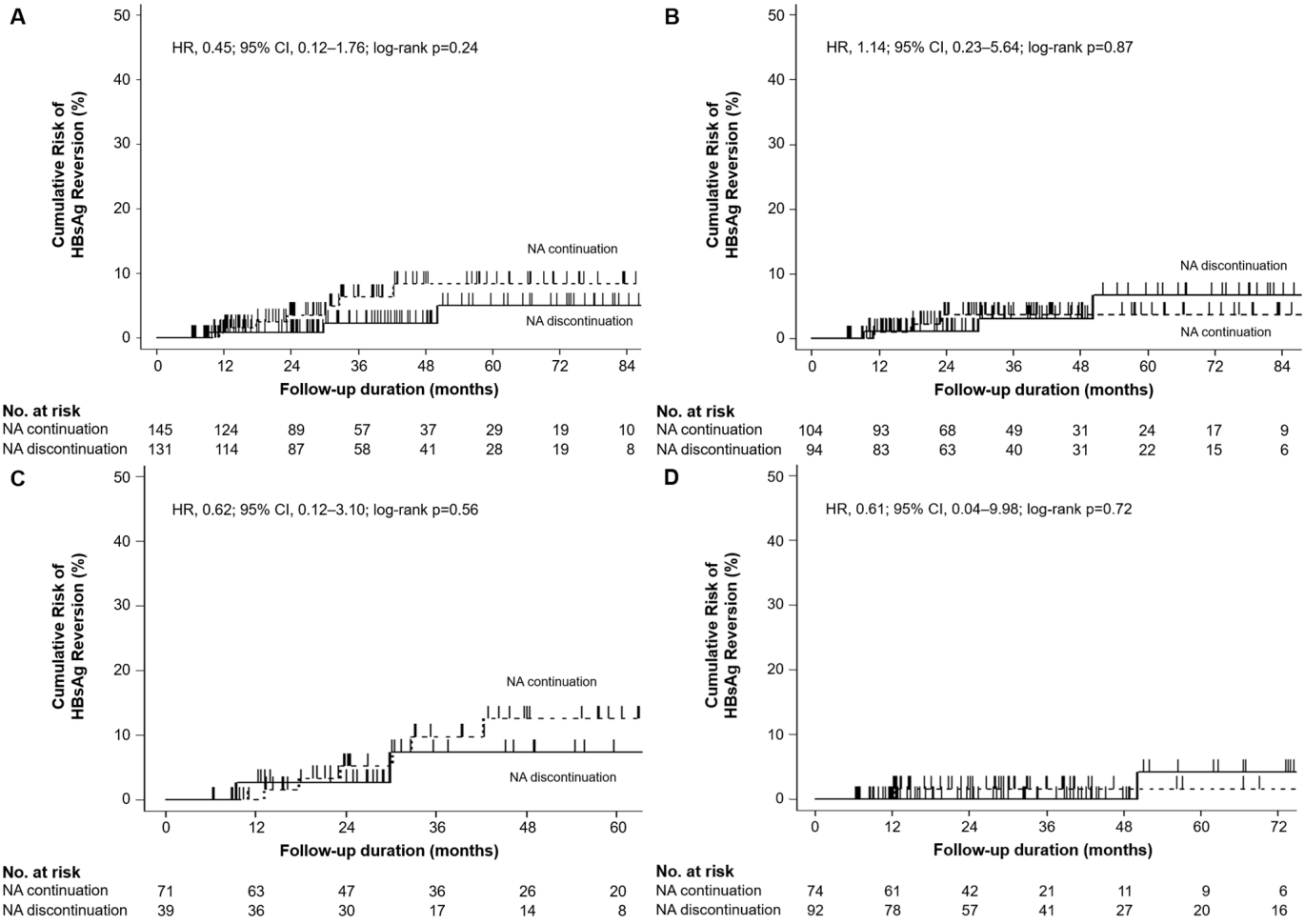

Figure 2 Kaplan-Meier estimates of cumulative risk of HBsAg reversion in (A) the entire study population (n=276), (B) the subgroup excluding patients who had a history of using two or more types of NAs (n=198), (C) patients with cirrhosis (n=110) and (D) patients without cirrhosis (n=166). CI, confidence interval; HBsAg, surface antigen of HBV; HR, hazard ratio; NA, nucleos(t)ide analogue.

clinical outcomes than NA continuation has not been proven. Thus, NA discontinuation was compared with NA continuation in the present study using a real-world large cohort from 16 multicentre databases in South Korea.

The results from the present study showed that patients in the NA discontinuation group had not significantly a higher risk of HBsAg reversion compared with the NA continuation group. This finding was consistent to the result from a recent pooled analysis of three international phase 3 trials that HBsAg loss after off-treatment of NAs or pegylated interferon-containing regimens was durable.[17] In the present study, the presence of cirrhosis was an independent risk factor for HBsAg reversion, which might be another novel finding. Meanwhile, HBsAg seroclearance may reflect a very low, but undetectable level of intrahepatic and/or serum HBV and both NA continuation and NA discontinuation may sustain HBsAg negativity through different mechanisms. NA continuation can continuously suppress low-level residual HBV replication.[18] Conversely, NA discontinuation may inhibit the reappearance of HBsAg by triggering host antiviral immunity.[19–21] In addition, maintaining NA after HBsAg seroclearance may not be beneficial when considering the economic burden and safety

Table 2 Univariable and multivariable analyses for HBsAg reversion

	Univariable			Multivariable		
	HR	95% CI	P value	aHR	95% CI	P value
Age ≥60 years	1.27	0.33 to 4.90	0.73			
Male	0.94	0.26 to 3.32	0.92			
Cirrhosis	4.98	1.06 to 23.51	0.04	5.99	1.27 to 28.31	0.02
ALT ≥40 IU/L	1.91	0.40 to 8.99	0.41			
Exposure to two or more types of NAs	2.05	0.58 to 7.30	0.27			
Positive anti-HBs	0.16	0.02 to 1.30	0.09	0.13	0.02 to 1.06	0.06
NA discontinuation	0.45	0.12 to 1.76	0.25	0.65	0.16 to 2.59	0.54

aHR, adjusted HR; ALT, alanine aminotransferase; anti-HBs, hepatitis B surface antibody; CI, confidence interval; HBsAg, surface antigen of HBV; HR, hazard ratio; NA, nucleos(t)ide analogue.

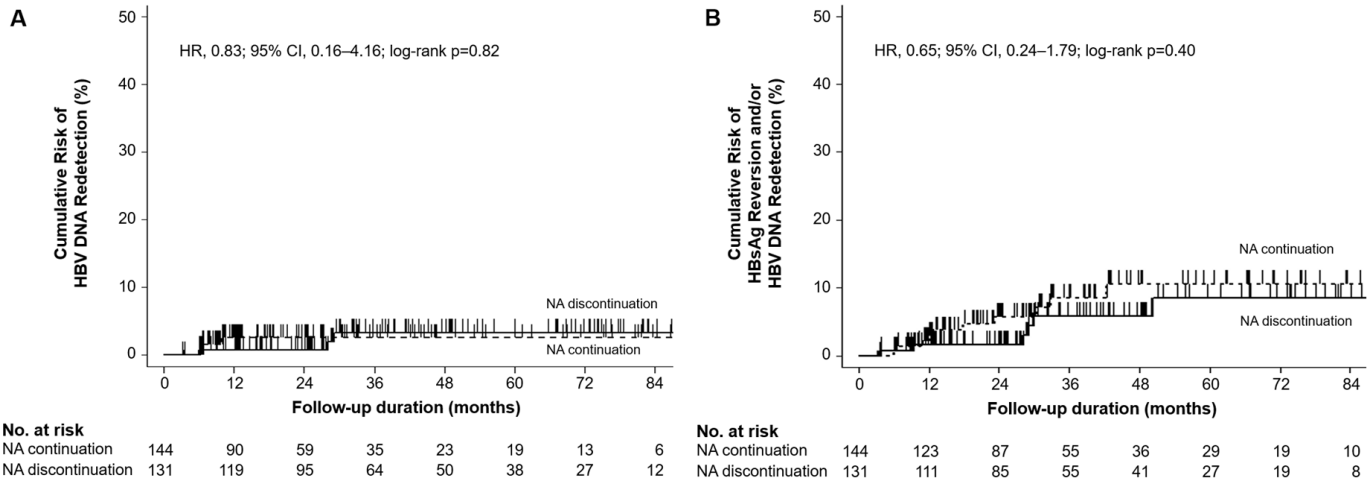

Figure 3 Kaplan-Meier estimates of cumulative risk of (A) HBV DNA redetection and (B) HBsAg reversion and/or HBV redetection in the entire study population (n=275, 1 missing). CI, confidence interval; HBsAg, surface antigen of HBV; HR, hazard ratio; NA, nucleos(t)ide analogue.

issues including possible adverse effects associated with long-term administration.[22 23]

In the present study, discordance was observed between positivity for HBsAg and HBV DNA tests. Although inclusion criteria included consecutive HBsAg negativity at least twice, seven patients who met the inclusion criteria showed detectable HBV DNA at the time of HBsAg seroclearance and were consequently excluded from the study. Notably patients with HBsAg reversion and patients with HBV DNA redetection did not overlap. Thus, regular monitoring with both serum HBsAg qualification test and HBV DNA quantification test might be warranted after HBsAg seroclearance. Although the majority of HBsAg reversion and HBV DNA redetection was transient, further studies with longer follow-up and more patients are warranted to elucidate whether both events are clinically insignificant. HBsAg reversion was set as the primary endpoint because we hypothesised HBsAg reversion would precede HBV DNA redetection. However, contrary to the expectations, these two endpoints occurred independently. When the composite endpoint (ie, either HBsAg reversion or HBV DNA redetection) was evaluated, intergroup difference was not observed. In addition, NAs inhibit HBV DNA polymerase and their long-term administration may lead to the selection of NA-resistant polymerase gene mutants,[24 25] which can be associated with mutations in the overlapping S gene.[26] However, even after excluding patients who experienced two or more types of NAs, who were presumed to have a NA-resistant P gene mutation that can result in false negativity of HBsAg, inferior outcomes of the NA discontinuation group were not maintained.

The present study has several strengths. This is the first study in which NA discontinuation and NA continuation were directly compared after NA-induced HBsAg seroclearance. Second, this was the largest real-world multicentre cohort study using rigorous inclusion and exclusion criteria to identify patients who

Table 3 Univariable and multivariable analyses for HBV DNA redetection

	Univariable			Multivariable		
	HR	95% CI	P value	aHR	95% CI	P value
Age ≥60 years	1.40	0.26 to 7.65	0.70			
ALT ≥40 IU/L	1.50	0.17 to 12.84	0.71			
Exposure to two or more types of NAs	14.86	1.73 to 127.57	0.01			
Positive anti-HBs	1.86	0.38 to 9.24	0.45			
NA discontinuation	0.83	0.16 to 4.16	0.82	0.66	0.12 to 3.50	0.62

aHR, adjusted HR; ALT, alanine aminotransferase; anti-HBs, hepatitis B surface antibody; CI, confidence interval; HR, hazard ratio; NA, nucleos(t)ide.

Table 4 Univariable and multivariable analyses for a composite endpoint (HBsAg reversion and/or HBV DNA redetection)

	Univariable			Multivariable		
	HR	95% CI	P value	aHR	95% CI	P value
Age ≥60 years	1.38	0.48 to 3.98	0.55			
Male	1.87	0.60 to 5.80	0.28			
ALT ≥40 IU/L	1.74	0.50 to 6.11	0.39			
Cirrhosis	1.24	0.46 to 3.32	0.66			
Exposure to two or more types of NAs	3.96	1.47 to 10.66	0.006			
Positive anti-HBs	0.53	0.17 to 1.64	0.27			
NA discontinuation	0.65	0.24 to 1.79	0.41	0.64	0.22 to 1.65	0.32

aHR, adjusted HR; ALT, alanine aminotransferase; anti-HBs, hepatitis B surface antibody; CI, confidence interval; HR, hazard ratio; NA, nucleos(t)ide.

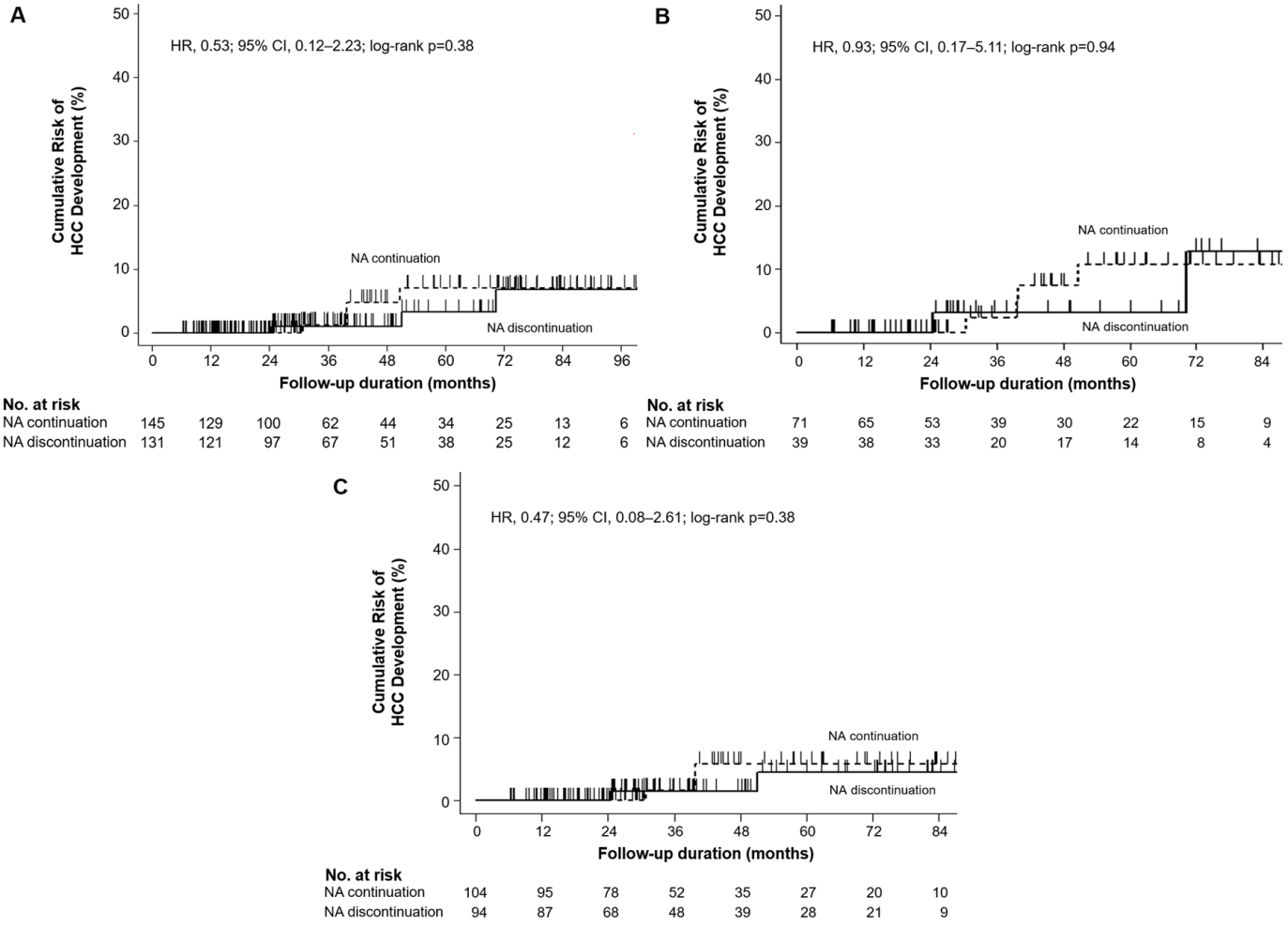

Figure 4 Kaplan-Meier estimates of cumulative risk of developing HCC in (A) the entire study population (n=276), (B) patients with cirrhosis (n=110) and (C) the subgroup excluding patients who had a history of using two or more types of NAs (n=198). CI, confidence interval; HBsAg, surface antigen of HBV; HCC, hepatocellular carcinoma; HR, hazard ratio; NA, nucleos(t)ide analogue.

achieved HBsAg seroclearance during NA treatment. Third, the definition of HBsAg seroclearance in the current study was more reliable than in previous studies because HBsAg negativity was confirmed at least twice with ≥6-month interval and patients with detectable serum HBV DNA were excluded regardless of HBsAg negativity.

Nevertheless, the present study has several limitations. First, this study was retrospective in design and immortal time bias and selection bias could have occurred. Immortal time bias could result from patients who were negative on the first HBsAg test and positive on the next HBsAg test ≥6 months from the first negative HBsAg date in the NA discontinuation group. However, no patient was excluded for this reason in the present study. Selection bias could have occurred when the number of excluded patients who received a HBsAg test only once and were not tested again was different between groups. However, there was no significant difference between the two groups (24 in the NA discontinuation group and 22 in the NA continuation group, p=0.63). Therefore, the risks of both immortal time bias and selection bias might not be significant in this study. Second, HBV genetic variations were not considered. Since almost HBV patients in South Korea are genotype C,[27] careful interpretation is needed and further studies involving other genotypes are warranted. Third, the number of patients who developed HCC was too small, thus, results should be interpreted with caution in that respect. Fourth, serum hepatitis B core-related antigen and HBV RNA are novel biomarkers that could be possibly associated with HBsAg reversion, HBV DNA redetection and HCC development, although they are not widely used in real-world practice because the corresponding assays are still research tools.[28 29] These biomarkers could not be analysed because stored blood samples were not available. Further prospective studies are warranted to assess the association with these viral markers.

In conclusion, the discontinuation of NA treatment was not associated with a higher risk of HBsAg reversion, HBV DNA redetection or HCC development compared with NA continuation among patients who achieved NA-induced HBsAg seroclearance. These results support that discontinuation of NAs could be recommended after HBsAg seroclearance confirmed on repeat testing at least 6 months later and HBsAg seroconversion is not required to discontinue NA treatment if a patient has achieved confirmed HBsAg seroclearance. Since cirrhosis and previous history of antiviral resistance might be associated with a higher risk for HBsAg reversion and HBV DNA redetection, respectively, those patients require more attention and further prospective studies with larger sample size are warranted.

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REFERENCES

1 World Health Organization. *Global hepatitis report 2017*. Geneva: World Health Organization, 2017.
2 Marcellin P, Gane E, Buti M, *et al*. Regression of cirrhosis during treatment with tenofovir disoproxil fumarate for chronic hepatitis B: a 5-year open-label follow-up study. *Lancet* 2013;381:468–75.
3 Yuen M-F, Seto W-K, Fung J, *et al*. Three years of continuous entecavir therapy in treatment-naïve chronic hepatitis B patients: viral suppression, viral resistance, and clinical safety. *Am J Gastroenterol* 2011;106:1264–71.
4 Chang T-T, Liaw Y-F, Wu S-S, *et al*. Long-term entecavir therapy results in the reversal of fibrosis/cirrhosis and continued histological improvement in patients with chronic hepatitis B. *Hepatology* 2010;52:886–93.
5 Hosaka T, Suzuki F, Kobayashi M, *et al*. Long-term entecavir treatment reduces hepatocellular carcinoma incidence in patients with hepatitis B virus infection. *Hepatology* 2013;58:98–107.
6 Wu C-Y, Lin J-T, Ho HJ, *et al*. Association of nucleos(t)ide analogue therapy with reduced risk of hepatocellular carcinoma in patients with chronic hepatitis B: a nationwide cohort study. *Gastroenterology* 2014;147:143–51.
7 Lampertico P, Invernizzi F, Viganò M, *et al*. The long-term benefits of nucleos(t)ide analogs in compensated HBV cirrhotic patients with no or small esophageal varices: A 12-year prospective cohort study. *J Hepatol* 2015;63:1118–25.
8 Papatheodoridis GV, Chan HL-Y, Hansen BE, *et al*. Risk of hepatocellular carcinoma in chronic hepatitis B: assessment and modification with current antiviral therapy. *J Hepatol* 2015;62:956–67.
9 Wang JP, Kao F-Y, Wu C-Y, *et al*. Nucleos(t)ide analogues associated with a reduced risk of hepatocellular carcinoma in hepatitis B patients: a population-based cohort study. *Cancer* 2015;121:1446–55.

10 Sarin SK, Kumar M, Lau GK, *et al*. Asian-Pacific clinical practice guidelines on the management of hepatitis B: a 2015 update. *Hepatol Int* 2016;10:1–98.
11 European Association for the Study of the Liver. EASL 2017 clinical practice guidelines on the management of hepatitis B virus infection. *J Hepatol* 2017;67:370–98.
12 Terrault NA, Lok ASF, McMahon BJ, *et al*. Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance. *Hepatology* 2018;67:1560–99.
13 Kim G-A, Lim Y-S, An J, *et al*. HBsAg seroclearance after nucleoside analogue therapy in patients with chronic hepatitis B: clinical outcomes and durability. *Gut* 2014;63:1325–32.
14 Yip TC-F, Wong GL-H, Wong VW-S, *et al*. Durability of hepatitis B surface antigen seroclearance in untreated and nucleos(t)ide analogue-treated patients. *J Hepatol* 2018;68:63–72.
15 Heimbach JK, Kulik LM, Finn RS, *et al*. AASLD guidelines for the treatment of hepatocellular carcinoma. *Hepatology* 2018;67:358–80.
16 Firth D. Bias reduction of maximum likelihood estimates. *Biometrika* 1993;80:27–38.
17 Lok AS, Zoulim F, Dusheiko G, *et al*. Durability of hepatitis B surface antigen loss with nucleotide analogue and peginterferon therapy in patients with chronic hepatitis B. *Hepatol Commun* 2020;4:8–20.
18 Wong DK-H, Seto W-K, Fung J, *et al*. Reduction of hepatitis B surface antigen and covalently closed circular DNA by nucleos(t)ide analogues of different potency. *Clin Gastroenterol Hepatol* 2013;11:1004–10.
19 Zimmer CL, Rinker F, Höner Zu Siederdissen C, *et al*. Increased NK Cell Function After Cessation of Long-Term Nucleos(t)ide Analogue Treatment in Chronic Hepatitis B Is Associated With Liver Damage and HBsAg Loss. *J Infect Dis* 2018;217:1656–66.
20 Höner Zu Siederdissen C, Rinker F, Maasoumy B, *et al*. Viral and Host Responses After Stopping Long-term Nucleos(t)ide Analogue Therapy in HBeAg-Negative Chronic Hepatitis B. *J Infect Dis* 2016;214:1492–7.
21 Jeng W-J, Chen Y-C, Chien R-N, *et al*. Incidence and predictors of hepatitis B surface antigen seroclearance after cessation of nucleos(t)ide analogue therapy in hepatitis B e antigen-negative chronic hepatitis B. *Hepatology* 2018;68:425–34.
22 Manns MP, Akarca US, Chang T-T, *et al*. Long-term safety and tolerability of entecavir in patients with chronic hepatitis B in the rollover study ETV-901. *Expert Opin Drug Saf* 2012;11:361–8.
23 Duarte-Rojo A, Heathcote EJ. Efficacy and safety of tenofovir disoproxil fumarate in patients with chronic hepatitis B. *Therap Adv Gastroenterol* 2010;3:107–19.
24 Park E-S, Lee AR, Kim DH, *et al*. Identification of a quadruple mutation that confers tenofovir resistance in chronic hepatitis B patients. *J Hepatol* 2019;70:1093–102.
25 Tenney DJ, Rose RE, Baldick CJ, *et al*. Long-term monitoring shows hepatitis B virus resistance to entecavir in nucleoside-naïve patients is rare through 5 years of therapy. *Hepatology* 2009;49:1503–14.
26 Zoulim F, Locarnini S. Hepatitis B virus resistance to nucleos(t)ide analogues. *Gastroenterology* 2009;137:1593–608.
27 Bae SH, Yoon SK, Jang JW, *et al*. Hepatitis B virus genotype C prevails among chronic carriers of the virus in Korea. *J Korean Med Sci* 2005;20:816–20.
28 Liu S, Zhou B, Valdes JD, *et al*. Serum hepatitis B virus RNA: a new potential biomarker for chronic hepatitis B virus infection. *Hepatology* 2019;69:1816–27.
29 Charre C, Levrero M, Zoulim F, *et al*. Non-Invasive biomarkers for chronic hepatitis B virus infection management. *Antiviral Res* 2019;169:104553.