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FULL LENGTH ARTICLE

# Durability of Hepatitis B surface antigen seroclearance in patients experienced nucleoside analogs or interferon monotherapy: A real-world data from Electronic Health Record

Zongqi Shi [a,b,1], Huizhi Zheng [a,1], Miaomiao Han [a,1], Jieli Hu [a], Yuan Hu [a], Xiaosong Li [a], Wenyan Zhu [c], Xinjun He [c], Haijun Deng [a,***], Quanxin Long [a,b,**], Ailong Huang [a,*]

[a] *Key Laboratory of Molecular Biology on Infectious Diseases, Ministry of Education, The Second Affiliated Hospital of Chongqing Medical University, Chongqing 400010, China*
[b] *Medical Data Research Institute of Chongqing Medical University, Chongqing 400010, China*
[c] *YiduCloud (Beijing) Technology Co.,Ltd, Beijing 100089, China*

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**Abstract** Little is known about the difference in durability of HBsAg seroclearance induced by nucleoside analogs (NAs) or by interferon (IFN). A real-world, retrospective cohort study was conducted. Patients were assigned into two groups: NAs monotherapy-induced HBsAg seroclearance subjects and IFN monotherapy induced-HBsAg seroclearance subjects. A total of 198 subjects, comprised by 168 NAs monotherapy-induced and 30 IFN monotherapy-induced, who achieved HBsAg seroclearance were included in this study. The one-year probabilities of confirmed HBsAg seroclearance were significantly different in patients with NAs monotherapy and IFN monotherapy (0.960 (with 95% CI 0.922–0.999) *vs.* 0.691 (with 95% CI 0.523–0.913), log-rank-$P$ = 4.04e-4). 73.3% (11 of 15) HBsAg recurrence occurred within one year after HBsAg seroclearance. The one-year probabilities of confirmed HBsAg seroclearance were higher in IFN monotherapy patients with anti-HBs than in IFN monotherapy patients without anti-HBs (0.839 (with 95% CI 0.657–1.000) *vs.* 0.489 (with 95% CI 0.251–0.953), log-rank test, $P$ = 0.024). Our

* Corresponding author.
** Corresponding author. Key Laboratory of Molecular Biology on Infectious Diseases, Ministry of Education, The Second Affiliated Hospital of Chongqing Medical University, Chongqing 400010, China.
*** Corresponding author.
*E-mail addresses:* pepper027@163.com (H. Deng), longqx@cqmu.edu.cn (Q. Long), ahuang@cqmu.edu.cn (A. Huang).
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[1] These authors contributed equally.

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Nucleoside analogs monotherapy

study thus provided novel insights into the durability of HBsAg seroclearance induced by NAs or IFN monotherapy. In particular, the HBsAg seroreversion rate was relatively high in IFN monotherapy subjects. The presence of anti-HBs was significantly correlated with a longer durability of functional cure induced by IFN treatment. And one-year follow-up in HBsAg seroclearance achieved individuals is proper for averting HBsAg seroreversion and other liver disease.


## Introduction

Chronic Hepatitis B virus (CHB) infection is still a serious global health issue. The World Health Organization (WHO) estimates that approximately 257 million people are infected with hepatitis B virus (HBV)) and carry a potential risk of adverse sequelae including cirrhosis and hepatocellular carcinoma. Hepatitis B surface antigen (HBsAg) seroclearance is a surrogate of immune control, and is considered as a 'function cure' endpoint.[1,2] The annual incidence of spontaneous HBsAg seroclearance is about 1%.[3,4] Patients who have achieved HBsAg seroclearance are often associated with favorable long-term clinical outcomes,[5–10] but challenges remain because only a small portion of these patients achieve HBsAg loss.[1] The rate of HBsAg seroclearance induced by treatment with nucleoside analogs (NAs) was considered as low as the spontaneous HBsAg seroclearance,[11–15] while interferon (IFN) treatment could achieve a slightly higher seroclearance rate than that induced by NAs.[16–20] Previous studies suggest that NAs induced-HBsAg seroclearance is as durable as spontaneous HBsAg seroclearance,[21] and is associated with improvement in liver histology and long-term clinical outcomes.[5,11] Nonetheless, it is not clear whether IFN monotherapy induced-HBsAg seroclearance, exhibits different durability of HBsAg seroclearance or different clinical outcomes compared with NAs-induced HBsAg seroclearance. In addition, there are debates on whether HBsAg loss alone or HBsAg loss accompanied with antibodies to HBsAg (HBsAg seroconversion) represents a better endpoint of therapy.[22]

It is difficult to establish a prospective cohort with proper size to analyze the characteristics and durability of HBsAg seroclearance, due to that HBsAg seroclearance, either achieved spontaneously or induced by treatment with NAs or IFN, is rarely observed in CHB patients.[14,15,18,23–26] Here, we investigated the characteristics of HBsAg seroclearance and the sustainability of response after HBsAg seroclearance by retrospectively examining the data of 198 patients with NAs or IFN monotherapy induced HBsAg seroclearance, which was mined from a data set containing more than 70,000 CHB patients and spanning more than 10 years.

## Materials and methods

### Study design and data source

A real-world retrospective cohort study was conducted by using data from the Integrated Clinical Data Systems of Chronic Hepatitis B (ICDS-CHB) of Chongqing Medical University, which contains more than 70,000 HBsAg positive individuals who visited the Second Affiliated Hospital of Chongqing Medical University between Jan 2006 and July 2021. The source data of chronic HBV patients were collected from an integrated clinical data system, which integrates Hospital Information System (HIS), Electronic Medical Record (EMR), Laboratory Information Management System (LIMS) and Radiology information system (RIS) in the Second Affiliated Hospital of Chongqing Medical University. To build the integrated system, the de-identified data items were restored, and transformed to standardized data in three steps (Extract, Transform and Load, ETL). Patient Index (PI) was used to identify the clinical cohort. Patients who met the following criteria were included: chronic HBV infection patients with at least two HBsAg test results, and with one HBsAg negative result; the achieved HBsAg seroclearance was induced by IFN or NAs monotherapy (HBsAg positive at the beginning, with clear therapy information in following data, discontinued IFN monotherapy or NAs monotherapy less than 5 years before HBsAg seroclearance) (Fig. 1). Patients who underwent liver transplantation, had any exposure to immunosuppressants and/or chemotherapeutic agents were excluded. Patients who underwent HBsAg vaccination during or after NAs or IFN monotherapy were excluded. Patients who had positive viral and/or serological markers for human immunodeficiency virus (HIV), hepatitis C virus (HCV) and/or hepatitis D virus (HDV) were also excluded. The study protocol was approved by the ethics committee of Chongqing Medical University.

### Data collection

Demographic data including gender and age were collected. Liver biochemistries, hematological and virologic parameters were also collected on each visit. Virologic parameters include HBV DNA, HBsAg (ARCHITECT®HBsAg Reagent Kit or Roche Elecsys® HBsAg II), anti-HBs (ARCHITECT®Anti-HBs Reagent Kit), HBeAg (ARCHITECT®HBeAg Reagent Kit), anti-HBe (ARCHITECT®Anti-HBe Reagent Kit), and anti-HBcAg (ARCHITECT®Anti-HBcIIReagent Kit). NAs-treated patients were defined as those prescribed with NAs for CHB, including lamivudine, adefovir dipivoxil, entecavir, telbivudine, and tenofovir disoproxil fumarate, in any treatment period. IFN-treated patients were defined as those prescribed with IFNs, including IFN $\alpha$-2a/2b or peginterferon (PEG-IFN) $\alpha$-2a/2b/$\lambda$-1a. Patients experienced combination therapy or sequential therapy of NAs and IFN were excluded from this study.

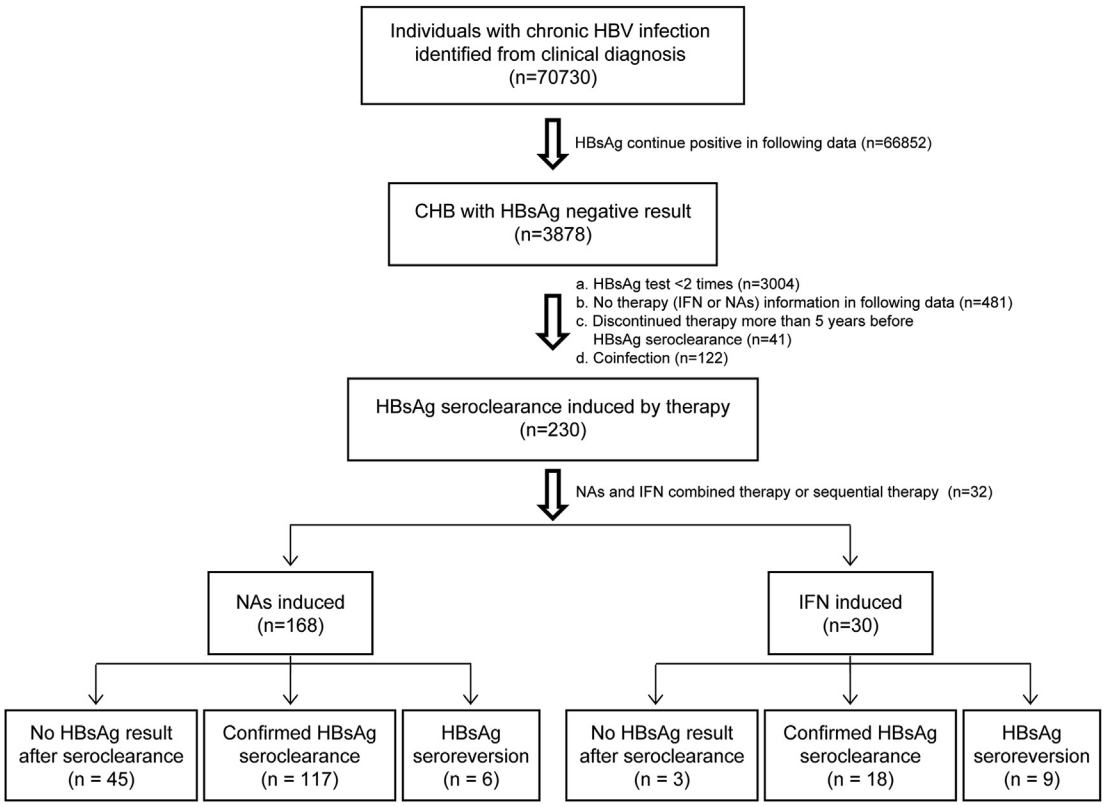


**Figure 1** Patient inclusion and exclusion flowchart. HBsAg, hepatitis B surface; HBV, hepatitis B virus; IFN, interferon; NAs, nucleos(t)ide analogue.

### Definition of events

CHB was defined as persistent HBV infection (the presence of detectable HBsAg in the blood or serum for longer than six months), with or without associated active viral replication and evidence of hepatocellular injury and inflammation. HBsAg seroclearance was defined as HBsAg results turning from positivity to negativity at once. Confirmed HBsAg seroclearance was defined as at least one more negative HBsAg result after HBsAg seroclearance with 6 months apart. HBsAg seroreversion was defined as reappearance of HBsAg after HBsAg seroclearance. HBsAg seroconversion was defined as detectable anti-HBs together with HBsAg negativity.

### Statistical analyses

Continuous variables with non-normal distribution were presented as median (interquartile range, IQR), and with normal distribution were presented as mean ± standard deviation, while categorical variables were presented as number (percentage). Qualitative differences between groups were compared by Fisher's exact test and quantitative differences between groups were compared by Student's *t* test or Mann Whitney *U* test as appropriate. The Kaplan—Meier's method was used to analyze the probabilities with 95% confidence interval (CI) of HBsAg seroclearance in patients with or without anti-HBs, and in patients with NAs or IFN monotherapy. Log-rank test was used to estimate the difference of probabilities of two groups. Univariate and multivariate Cox proportional hazards (PH) regression model was used to estimate the independent factor for HBsAg seroclearance durability. All statistical tests were two-sided and *P* value of less than 0.05 was considered statistically significant. Statistical analyses were performed using R software, version 3.6.0.

## Results

### Clinical characteristics at time of therapy initiation

Our database included 70,730 subjects with at least one positive HBsAg test, 64.1% of the individuals was male and 35.9% was female, and the age distribution was ranged from 1 to 98 years with median age of 42 years (IQR, 31—52). Among these patients, 3878 subjects had HBsAg negative results. After excluding patients with incomplete data, or coinfected with HCVor HIV, or exposed to immunosuppressants and/or chemotherapeutic agents, or experienced combination or sequential therapy of NAs and IFN, 198 patients with HBsAg seroclearance were included in the final analysis, including 168 subjects from 22,289 patients with NAs monotherapy and 30 subjects from 908 patients with IFN monotherapy (0.75% *vs.* 3.3%, $P < 0.001$) (Fig. 1).

The NAs or IFN monotherapy patients had comparable gender distribution, HBeAg (positive or negative), alanine aminotransferase (ALT) level (normal or abnormal) and HBV DNA level at the time of their therapy initiation. The age of NAs monotherapy patients was noticeably elder than IFN monotherapy patients (40.3 ± 12.9 years *vs.* 30.9 ± 9.5 years, $P < 0.001$) (Table 1). Among the 168 NAs monotherapy patients, 24, 19, 44, 87 and 9 cases had received lamivudine,

**Table 1** Baseline clinical characteristics of the 198 CHB patients who had HBsAg seroclearance with NAs or IFN therapy.

| Clinical | NAs ($n$ = 168) | IFN ($n$ = 30) | $P$-value |
|---|---|---|---|
| Age (mean ± sd) | 40.3 ± 12.9 | 30.9 ± 9.5 | <0.001 |
| Sex | | | 0.838 |
| Male | 106 (63.1%) | 20 (66.7%) | |
| Female | 62 (36.9%) | 10 (33.3%) | |
| HBV DNA | | | 0.413 |
| Positive | 73 (43.5%) | 16 (53.3%) | |
| Negative | 81 (48.2%) | 12 (40.0%) | |
| Missing | 14 (8.3%) | 2 (6.7%) | |
| HBsAb | | | 1.000 |
| Positive, ≥10 mIU/ml | 5 (3.0%) | 0 (0.0%) | |
| Negative, <10 mIU/ml | 146 (86.9%) | 20 (66.7%) | |
| Missing | 17 (10.1%) | 10 (33.3%) | |
| HBsAb positive titers | | | 1.000 |
| Low, 10–100 mIU/ml | 1 (0.6%) | 0 (0.0%) | |
| Intermediate, 100–1000 mIU/ml | 4 (2.4%) | 0 (0.0%) | |
| HBcAb | | | 1.000 |
| Positive, ≥1 COI | 9 (5.4%) | 1 (3.3%) | |
| Negative, <1 COI | 135 (80.4%) | 19 (63.3%) | |
| Missing | 24 (14.3%) | 10 (33.3%) | |
| HBeAg | | | 1.000 |
| Positive, ≥1 COI | 67 (39.9%) | 10 (33.3%) | |
| Negative, <1 COI | 81 (48.2%) | 13 (43.3%) | |
| Missing | 20 (11.9%) | 7 (23.3%) | |
| HBeAb | | | 1.000 |
| Positive, <1 COI | 82 (48.8%) | 12 (40.0%) | |
| Negative, ≥1 COI | 62 (36.9%) | 8 (26.7%) | |
| Missing | 24 (14.3%) | 10 (33.3%) | |
| ALT | | | 0.414 |
| Normal, <35 U/L | 48 (28.6%) | 7 (23.3%) | |
| Abnormal, ≥35 U/L | 97 (57.7%) | 9 (30.0%) | |
| Missing | 23 (13.7%) | 14 (46.7%) | |
| ALT, U/L | 56.0 (29.0–236.0) | 63.0 (29.2–214.8) | 0.724 |
| AST, U/L | 57.0 (26.5–204.5) | 45.5 (33.2–98.0) | 0.389 |
| Missing | 52 (31.0%) | 14 (46.7%) | |

telbivudine, adefovir, entecavir and tenofovir before HBsAg seroclearance, respectively. Some patients had received more than one type of NAs. Among the 30 IFN monotherapy patients, 19, 2, 6 and 3 cases had received PEG-IFN -α2a, PEG-IFN -α2b, recombined human IFN-α1b and recombined human IFN-α2b therapy, respectively.

## Clinical characteristics of subjects who achieved HBsAg seroclearance

Therapy duration and time needed for HBsAg seroclearance during therapy were analyzed as priority. Firstly, the median time from therapy initiation to HBsAg seroclearance for the NAs monotherapy cohort was longer than the IFN monotherapy cohort (20.8 months (IQR, 6.5–48.0) *vs.* 15.0 months (IQR, 9.4–33.6), $P = 0.723$), although the difference was not statistically significant. Secondly, the median duration time of NAs monotherapy was longer than IFN monotherapy (12.1 months (IQR, 3.7–33.2) *vs.* 7.9 months (IQR, 5.8–12.4), $P = 0.153$). Thirdly, higher percentage of HBsAg seroclearance occurred during treatment in the NAs monotherapy cohort than the IFN monotherapy cohort (48.8% *vs.* 30.0%, $P = 0.073$) (Table 2).

At the time of HBsAg seroclearance, 38.1% (64 of 168) of NAs monotherapy individuals developed concomitant anti-HBs, which had no statistical difference compared with IFN monotherapy individuals (23.3%, 7 of 30). In anti-HBs positive individuals, the quantitative value of anti-HBs in NAs monotherapy individuals was lower than that in IFN monotherapy individuals (55.1 mIU/mL (IQR, 22.5–284.2) *vs.* 166.6 mIU/mL (IQR, 120.6–513.8), $P = 0.070$), and 62.5% (40 of 64) of NAs monotherapy individuals had low anti-HBs titers (10–100 mIU/mL), while 71.4% (5 of 7) of IFN monotherapy individuals had moderate anti-HBs titers (100–1000 mIU/mL). The proportion of individuals with normal ALT level in the NAs-monotherapy cohort was higher than that in the IFN-monotherapy cohort (78.6% *vs.* 40.0%, $P = 0.007$). The quantitative of ALT and AST levels in the NAs monotherapy individuals were significantly lower than that in IFN monotherapy individuals (21.0 U/L *vs.* 34.0 U/L, $p = 0.002$ and 24.0 U/L *vs.* 27.0 U/L, $p = 0.013$, respectively) (Table 2).

Furthermore, the relationship between laboratory testing results at baseline and time needed for HBsAg seroclearance was analyzed in NAs or IFN monotherapy individuals with HBsAg seroclearance. HBeAg positive patients had shorter HBsAg clearance time than HBeAg negative patients in both NAs monotherapy patients (median 13.5 months (IQR, 5.5–39.2) *vs.* 22.9 months (IQR, 8.0–48.1), $P = 0.205$) and IFN-induced HBsAg seroclearance patients (median 11.6 months (IQR, 6.7–13.4) *vs.* 21.6 months (IQR, 12.4–36.0), $P = 0.154$). Patients with abnormal ALT level at baseline also achieved HBsAg seroclearance faster than patients with normal ALT level in NA-monotherapy cohort (median 6.4 months (IQR, 2.1–28.3) *vs.* 16.9 months (IQR, 6.2–31.3), $P = 0.009$), while this was not observed in IFN-monotherapy cohort (median 6.5 months (IQR, 5.2–12.1) *vs.* 7.2 months (IQR, 6.3–11.9), $P = 0.408$).

## Clinical outcomes after HBsAg seroclearance induced by NAs or IFN monotherapy

Of the 168 HBsAg seroclearance individuals with NAs monotherapy, 123 were followed up after HBsAg seroclearance, while 27 of the 30 IFN-experienced HBsAg seroclearance individuals were followed up. In the 123 patients with HBsAg seroclearance induced by NAs, 41, 12 and 4 patients developed liver cirrhosis, hepatocellular carcinoma (HCC) and hepatic failure/hepatic encephalopathy, respectively during our observation. Six of the 123 patients had HBsAg seroreversion in 6.6 months (IQR, 4.9–20.2), among whom one developed liver cirrhosis and HCC at the HBsAg seroreversion point, and another one developed liver cirrhosis

**Table 2** Clinical characteristics at the time of HBsAg seroclearance of 198 CHB patients who had HBsAg seroclearance with NAs or IFN therapy.

| Clinical | NAs ($n$ = 168) | IFN ($n$ = 30) | $P$-value |
|---|---|---|---|
| HBsAg duration (months) | 20.8 (6.5—48.0) | 15.0 (9.4—33.6) | 0.723 |
| Treatment duration (months) | 12.1 (3.7—33.2) | 7.9 (5.8—12.4) | 0.153 |
| Follow up status | | | <0.001 |
| Confirmed HBsAg seroclearance | 117 (69.6%) | 18 (60.0%) | |
| HBsAg seroreversion | 6 (3.6%) | 9 (30.0%) | |
| No HBsAg result after seroclearance | 45 (26.8%) | 3 (10.0%) | |
| Follow up times | 2 (1—4) | 3 (1—6) | 0.092 |
| Follow up (months) | 13.2 (6.9—26.5) | 19.3 (10.0—35.0) | 0.127 |
| Duration of HBsAg seroclearance (months) | 12.1 (6.5—25.2) | 13.3 (6.2—35.0) | 0.858 |
| Treatment cessation before/after HBsAg seroclearance | | | 0.073 |
| Before | 86 (51.2%) | 21 (70.0%) | |
| After | 82 (48.8%) | 9 (30.0%) | |
| HBsAb | | | 0.379 |
| Positive, ≥10 mIU/ml | 64 (38.1%) | 7 (23.3%) | |
| Negative, <10 mIU/ml | 103 (61.3%) | 18 (60.0%) | |
| Missing | 1 (0.6%) | 5 (16.7%) | |
| HBsAb positive titers | | | 0.029 |
| Low, 10—100 mIU/ml | 40 (62.5%) | 1 (14.3%) | |
| Intermediate, 100—1000 mIU/ml | 19 (29.7%) | 5 (71.4%) | |
| Hgh, >1000 mIU/ml | 5 (7.8%) | 1 (14.3%) | |
| HBsAb positive titers | 55.1 (22.5—284.2) | 166.6 (120.6—513.8) | 0.070 |
| HBcAb | | | 0.743 |
| Negative, ≥1 COI | 20 (11.9%) | 2 (6.7%) | |
| Positive, <1 COI | 141 (83.9%) | 23 (76.7%) | |
| Missing | 7 (4.2%) | 5 (16.7%) | |
| HBeAg | | | 0.088 |
| Positive, ≥1 COI | 5 (3.0%) | 3 (10.0%) | |
| Negative, <1 COI | 158 (94.0%) | 24 (80.0%) | |
| Missing | 5 (3.0%) | 3 (10.0%) | |
| HBeAb | | | 0.626 |
| Positive, <1 COI | 119 (70.8%) | 20 (66.7%) | |
| Negative, ≥1 COI | 42 (25.0%) | 5 (16.7%) | |
| Missing | 7 (4.2%) | 5 (16.7%) | |
| ALT | | | 0.007 |
| Normal, <35 U/L | 132 (78.6%) | 12 (40.0%) | |
| Abnormal, ≥35 U/L | 33 (19.6%) | 11 (36.7%) | |
| Missing | 3 (1.8%) | 7 (23.3%) | |
| ALT, U/L | 21.0 (15.0—31.0) | 34.0 (22.5—54.0) | 0.002 |
| AST, U/L | 24.0 (19.0—30.5) | 27.0 (24.5—40.0) | 0.013 |
| Missing | 9 (5.4%) | 7 (23.3%) | |

after HBsAg seroreversion. In the 27 patients with HBsAg seroclearance induced by IFN, 9 patients had HBsAg seroreversion in 7.0 months (IQR, 5.8—10.5). No patients developed liver cirrhosis or HCC during follow-up in the IFN monotherapy patients.

## Durability of HBsAg seroclearance

The gender and age distribution in the 123 NAs monotherapy and 27 IFN monotherapy induced HBsAg seroclearance individuals with followed-up visit records showed no statistical difference. During follow-up after HBsAg seroclearance, serum HBsAg was checked for a median (IQR) of 3 (2—5) times at a median (IQR) interval of 16.3 (7.3—30.2) months in NAs monotherapy patients, and was checked for 4 (2—7) times at an interval of 19.3 (10.0—35.0) months in IFN monotherapy patients. In the follow-up, 6 of 123 (4.9%) HBsAg seroclearance patients in the NAs monotherapy group showed HBsAg recurrence, while 9 of 27 (33.3%) HBsAg seroclearance patients in the IFN monotherapy group showed HBsAg recurrence. The HBsAg seroreversion rate in NAs monotherapy patients was significantly lower than IFN monotherapy patients ($p < 0.001$) (Table 2). All the patients who experienced HBsAg seroreversion had no recurrence of positive HBV DNA, and both groups had one patient with anti-HBs positive. Detailed information of the patients with recurrence of HBsAg were summarized in Table S1 (IFN monotherapy) and Table S2 (NAs monotherapy). The one-year and three-year probability of confirmed HBsAg seroclearance in

patients with NAs therapy was significantly higher than that in patients with IFN therapy (one-year: 0.960 (with 95% CI 0.922—0.999) *vs*. 0.691 (with 95% CI 0.523—0.913), log-rank-*P* = 4.04e-4; three-year: 0.931 (with 95% CI 0.867—1) *vs*. 0.641 (with 95% CI 0.468—0.879)) (Fig. 2A).

Factors associated with HBsAg seroreversion were further analyzed by Cox PH regression modeling. Based on the univariate Cox PH regression model, the estimated hazard ratio (HR) of NAs monotherapy for the HBsAg seroreversion was 0.152 (with 95% CI 0.054—0.428, *P* = 3.66e-4), patients with age >35 years had a lower risk of HBsAg seroreversion (HR = 0.284, with 95% CI 0.090—0.900, *P* = 0.032), while patients with abnormal ALT had a higher risk of HBsAg seroreversion (HR = 3.862, with 95% CI 1.290—11.567, *P* = 0.016). After adjusting for ALT and age in a multivariate Cox PH regression model, therapeutic method was still significantly associated with HBsAg seroreversion (HR of NAs therapy was 0.228, with 95% CI 0.058—0.888, *P* = 0.033) (Fig. 2B, Table S3), suggesting that the therapeutic method was an independent factor of the HBsAg seroreversion.

The time to HBsAg seroreversion in patients with NAs monotherapy was similar to that in IFN monotherapy group (6.6 months (IQR, 4.8—20.2) *vs*. 7.0 months (IQR, 5.8—10.5), *P* = 1.000). In detail, of the 15 HBsAg seroreversion patients, 73.3% (11 of 15) of them occurred within the first year after seroclearance, and 13.3% (2 of 15) occurred during the 2nd year after HBsAg seroclearance. These results suggest that one-year follow-up is sufficient for HBsAg monitoring for most patients since very few patients experienced seroreversion after one year.

In addition, we explored the role of HBsAg seroconversion in the durability of HBsAg seroclearance. The proportion of the patients with positive anti-HBs at the HBsAg

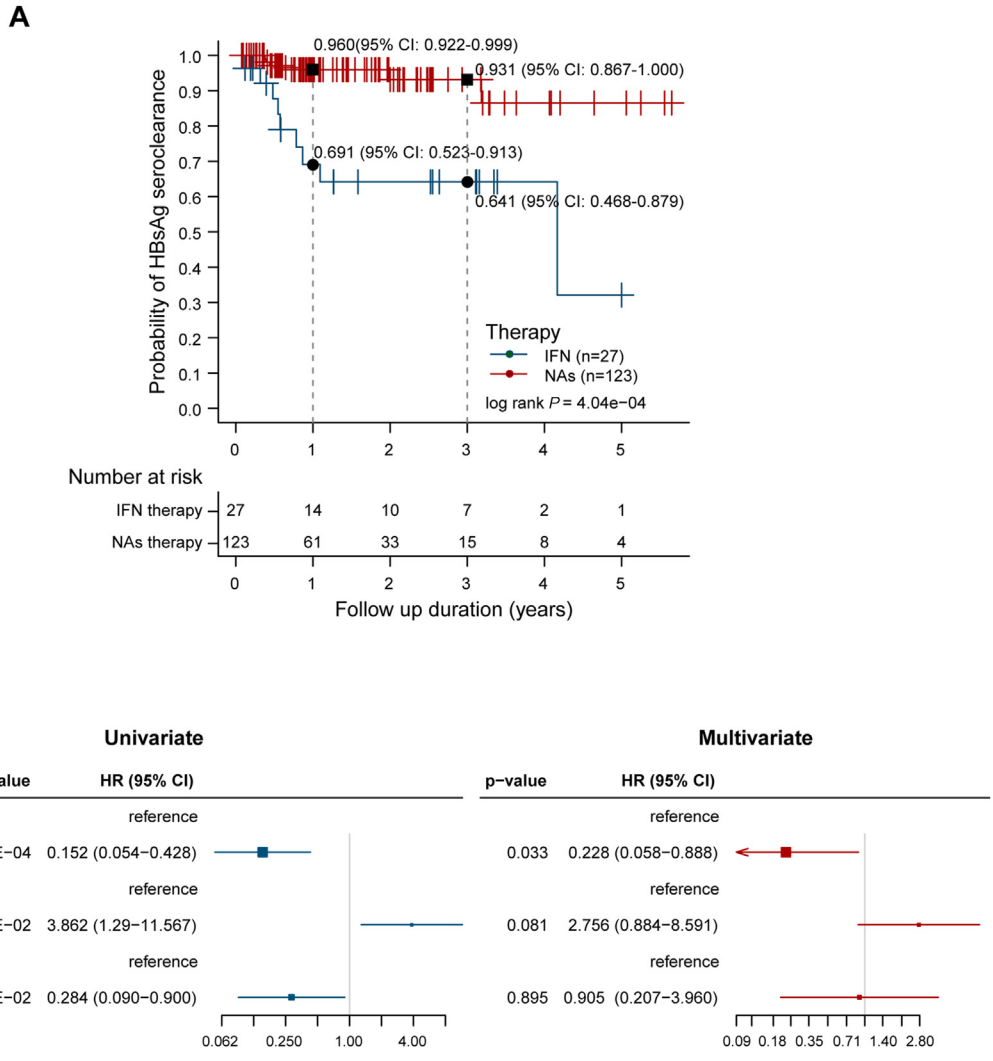


**Figure 2** Analysis of HBsAg seroclearance of NAs and IFN monotherapy. (**A**) Kaplan—Meier analysis of confirmed NAs monotherapy induced and IFN monotherapy induced HBsAg seroclearance (NAs monotherapy, *n* = 123; IFN monotherapy, *n* = 27). (**B**) Univariate and Multivariate Cox regression model of the clinical characteristics. Probability of HBsAg seroclearance of NAs and IFN monotherapy was calculated by Kaplan—Meier method, and statistical significance was measured by log-rank test. Univariate and multivariate Cox proportional hazards regression model was used to estimate the independent factor for HBsAg seroclearance durability. HBsAg, hepatitis B surface antigen; IFN, interferon; NA, nucleos(t)ide analogue.

seroclearance time point (47/123, 38.2% *vs.* 6/27, 22.2%, $P = 0.127$), within one year (74/123, 60.2% *vs.* 14/27, 51.9%, $P = 0.518$), and at any follow-up point after HBsAg seroclearance (93/123, 76.1% *vs.* 15/27, 55.6%, $P = 0.056$) showed no difference between NAs monotherapy and IFN monotherapy. The median time from initial HBsAg seroclearance to development of anti-HBs were 10.6 months (IQR, 6.4–15.4) and 4.7 months (IQR, 3.9–6.4) ($P = 0.013$) in NAs monotherapy and IFN monotherapy group, respectively. For patients in IFN monotherapy group, the one-year probabilities of confirmed HBsAg seroclearance were different in patients with and without anti-HBs (0.825 (with 95% CI 0.631–1.000) *vs.* 0.539 (with 95% CI 0.306–0.951), log-rank test, $P = 0.051$, Fig. 3A), and at any time during follow-up (0.839 (with 95% CI 0.657–1.000) *vs.* 0.489 (with 95% CI 0.251–0.953), log-rank test, $P = 0.024$, Fig. 3B). However, this difference was not observed between patients with and without HBsAg seroconversion in NAs monotherapy patients (within one year log-rank $p = 0.858$, Fig. 3C) at any time during follow-up (log-rank test, $P = 0.553$, Fig. 3D).

## Discussion

HBsAg seroclearance, representing a 'functional cure', in CHB patients is not a common event. Durability of HBsAg seroclearance and whether anti-HBs is necessary for maintenance of HBsAg seroclearance in NA or IFN monotherapy experienced CHB patients are still in debate.[22] A large HBsAg seroclearance cohort over a long period of clinical follow-up is ideal to evaluate the characteristics of patients who have achieved functional cure, which is hard to achieve in a well-controlled and regulated clinical trial. Previous study has employed electronic medical informatics data to describe clearance dynamics of HBsAg and HBeAg in CHB patients,[27] while our study collected CHB treatment data using an algorithmic approach from the Integrated Clinical Data Systems of Chronic Hepatitis B (ICDS-CHB) of Chongqing Medical University, which contains more than 70,000 HBsAg positive individuals who visited the Second Affiliated Hospital of Chongqing Medical University between Jan 2006 and July 2021. From this integrated database, HBsAg seroconversion in CHB patients with antiviral experience (NAs or IFN) was retrieved. Demographic information, HBV related laboratory test results, and medical treatment were extracted for further analysis. To our knowledge, this is the first study to compare the HBsAg seroclearance durability induced by NAs and IFN monotherapy by employing big medical data from real world and tracking longitudinal data in an unbiased way.

In line with previous studies,[11,21] the HBsAg seroclearance rate in IFN monotherapy subjects was significantly higher than NAs monotherapy subjects (0.75% *vs.* 3.3%, $P < 0.001$). Previous studies described a similar HBsAg seroreversion rate with spontaneous or NAs induced subjects, which usually included NAs and IFN combination or sequential therapy subjects.[28,29] Here, we found that HBsAg seroclearance induced by NAs was durable, and the rate of HBsAg recurrence rate at one year and two years were 3.3% and 4.1%, respectively. In addition, we found that the HBsAg recurrence rates were 25.9% and 29.6% at one year and two years after seroclearance in the IFN monotherapy cohort, significantly higher than NA monotherapy cohort (Fig. 2). Recent studies have shown a significant improvement in HBsAg-negative rate when IFN and NAs combination or switch-to/add-on IFN therapy is used in patients under long-term NAs therapy,[29,30] with a comparable HBsAg seroclearance durability rate compared with NAs monotherapy induced or untreated cohort.[21,26,29]

IFN has been shown to have both antiviral, by altering the epigenetic state of Covalent Closed Circular DNA (cccDNA) mini chromosomes,[31–34] and immunomodulatory effects, by activating HBV-specific proliferation and T cell responses.[35] HBsAg seroclearance durability in IFN monotherapy subjects is lower than NAs monotherapy subjects. A plausible explanation to this is that HBsAg seroclearance in IFN monotherapy subjects is not due to the complete elimination of cccDNA but rather a prolonged suppression of cccDNA transcription, upon the cessation of IFN therapy, the silenced cccDNA would go back to a transcriptional resurgence state in some subjects. On the other side, the median IFN therapy duration in current study was 7.9 months (IQR, 5.8–12.4), which is shorter than the recommend standard 48 weeks IFN regimen,[10,36] may be caused by the drug compliance in therapy from real world. Previous studies also described higher HBsAg seroreversion in patients experienced 16 weeks IFN combination therapy than HBsAg seroclearance patients experienced longer IFN interventions.[30,37] Thereafter, the relative higher HBsAg seroreversion rate in patients induced by IFN monotherapy also need to be carefully interpreted with the IFN treatment duration.

Another important issue is whether anti-HBs contributes to the durability of HBsAg seroclearance. Anti-HBs positivity has been reported to be associated with a decreased risk of reactivation in patients with resolved HBV receiving chemotherapy for hematological malignancies without antiviral prophylaxis.[38] Another cohort study showed that the presence of anti-HBs was not essential for maintaining HBsAg seroclearance after NAs treatment.[21] In line with these observations, we found no statistical difference in the probability of confirmed HBsAg seroclearance in NAs monotherapy patients with or without anti-HBs at the time of HBsAg seroclearance and within one year of HBsAg seroclearance. In another study including spontaneous, NAs-treated and IFN-treated HBsAg seroclearance, the HBsAg seroreversion rate in those who were anti-HBs negative was numerically twice compared to those who were anti-HBs positive, but without statistical difference.[26] In our study, the probability of confirmed HBsAg seroclearance among IFN monotherapy induced subjects with anti-HBs was significantly higher than those without anti-HBs, in line with results from 48 or 96 weeks following-up after HBsAg seroclearance,[39,40] indicating that anti-HBs could be a potential marker to indicate the durability of HBsAg seroclearance in IFN therapy subjects, which is expected to be validated in further study.

The main limitation of this analysis is the potential for bias in the data from residual confounding,[41] which may raise from the variation of the visit interval of CHB patients (may affect the calculation of HBsAg seroclearance time

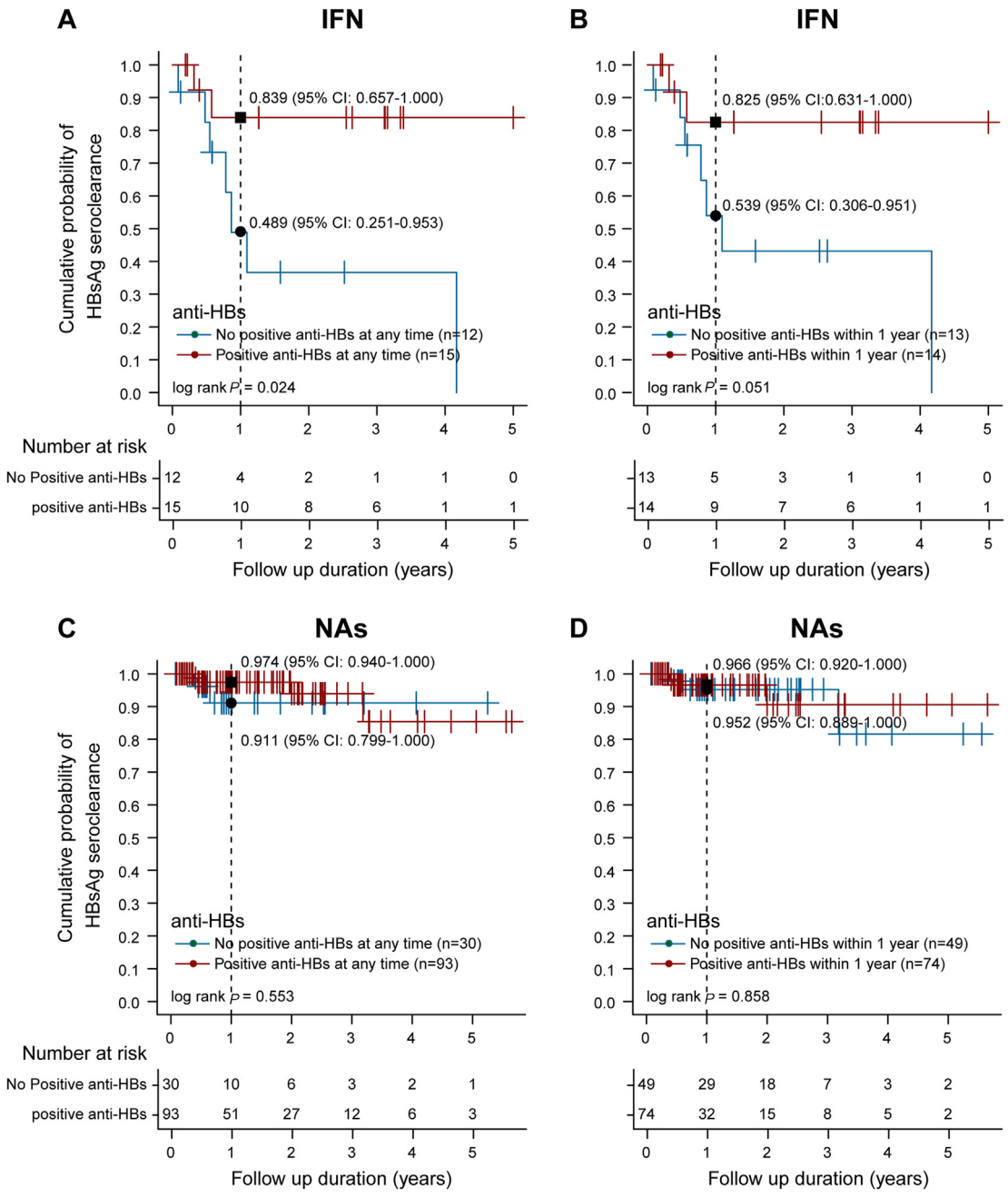


**Figure 3** Kaplan—Meier analysis of confirmed IFN monotherapy induced (**A, B**) and NA monotherapy induced (**C, D**) HBsAg seroclearance with or without anti-HBs. Probability of HBsAg seroclearance of groups were calculated by Kaplan—Meier method. Statistical significance was measured by log-rank test.

and therapy duration time), incomplete visit record (high availability of NAs also may lead to incomplete visit record in patients). Data included in this study are from a single center, which may limit generalizability to other centers. Besides, HBV genotype is not included in current study, the fact that some viral markers were detected by more economic rather than by the most advanced methods (HBsAg level routinely detected by semiquantitative method, which could not tell the HBsAg kinetics during therapy; the HBV DNA detection limit is 100 IU/ml), may also lead to missing of some information.

## Ethics declaration

The study was approved by the Ethics Committee of Chongqing Medical University.

## Author contributions

Quanxin Long, Haijun Deng and Ailong Huang conceived and designed the study. Zongqi Shi had full access to the original data and collected the data. Wenyan Zhu and Xinjun He established the integrated clinical data system. Zongqi Shi,

Haijun Deng, Huizhi Zheng, Miaomiao Han and Yuan Hu analyzed the data. Quanxin Long and Haijun Deng wrote the manuscript. Jieli Hu and Xiaosong Li revised the manuscript.

## Conflict of interests

Authors Wenyan Zhu and Xinjun He are employed by Yidu-Cloud (Beijing) Technology Co.,Ltd. As a Co-Editor in Chief of Genes & Diseases, the corresponding author Prof. Ailong Huang was blinded from reviewing or making decisions on this manuscript. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2022.03.003.

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