Original Article

Hepatic flares promote rapid decline of serum hepatitis B surface antigen (HBsAg) in patients with HBsAg seroclearance: A long-term follow-up study

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Aim: Serum hepatitis B surface antigen (HBsAg) seroclearance is one of the ultimate goals of management of chronic hepatitis B. We investigated the kinetics of serum HBsAg before HBsAg seroclearance in patients with chronic hepatitis B.

Methods: We retrospectively analyzed 392 Japanese chronic hepatitis B patients who had been followed for 5 years or more between 1980 and 2000. Serum HBsAg levels were measured annually using chemiluminescent enzyme immunoassay.

Results: During a median follow up of 14 years, 50 patients demonstrated HBsAg seroclearance (annual incidence rate, 0.91%). Multivariate analysis with baseline characteristics revealed that HBsAg of less than 3.3 log IU/mL (hazard ratio [HR], 2.22; $P = 0.008$) and treatment with nucleoside/nucleotide analog (HR, 0.12; $P = 0.001$) were independent predictive factors for seroclearance. The median HBsAg levels at 20, 10, 5, 3 and 1 year prior to seroclearance were 3.89, 2.84, 1.84, 0.78 and −1.10 log IU/mL, respectively. The rapid decline group, comprising patients who achieved HBsAg seroclearance within 5 years after confirmed HBsAg levels of 2 log IU/mL, demonstrated: (i) high alanine aminotransferase (ALT) levels; and (ii) a low frequency of liver cirrhosis progression. A significant reduction in annual HBsAg levels was found in years marked by at least one ALT flare (ALT ≥200 IU/L) (flare [+], $n = 62$) than in those without (flare [−], $n = 323$) (0.29 vs 0.17 log IU/mL/year, $P = 0.003$).

Conclusion: Hepatic flares promoted rapid declines and greater annual reductions of HBsAg levels in patients with HBsAg seroclearance.

Key words: alanine aminotransferase flare, chronic hepatitis B, hepatitis B surface antigen seroclearance, hepatitis B surface antigen quantification

INTRODUCTION

HEPATITIS B VIRUS (HBV) infection is a worldwide health problem affecting an estimated 350 million people. Chronic HBV infection confers an increased risk of liver cirrhosis (LC), liver failure and hepatocellular carcinoma (HCC).[1] Hepatitis B surface antigen (HBsAg)

seroclearance during the natural course of chronic HBV infection is associated with favorable long-term outcomes,[2,3] although the development of HCC remains possible.[4–6] The annual incidence of HBsAg seroclearance identified in various studies has varied widely, from 0.5% to 2.26%/year, depending on enrollment criteria.[7–9] The recent development of serum HBsAg quantification has enabled us to predict the response to pegylated interferon (PEG IFN) treatment for chronic hepatitis B,[10,11] and clarified the natural history of chronic HBV infection.[12] Recent longitudinal studies from Asia demonstrated that lower HBsAg levels and greater annual reductions independently predicted HBsAg seroclearance.[12–14] These longitudinal studies were limited by the lack of long-term serial changes in HBsAg levels. In this study, the primary end-point was

the kinetics of serum HBsAg levels in long-term follow up (median follow-up duration, 17.5 years) of patients demonstrating HBsAg seroclearance. We demonstrated different patterns of HBsAg decline prior to HBsAg seroclearance. As the secondary end-point, we investigated the factors associated with variations in serum HBsAg kinetics, which indicated the importance of alanine aminotransferase (ALT) flare. Finally, we investigated the relationship between annual reductions of HBsAg levels and ALT flare.

METHODS

Patients

FROM 1980 TO 2000, 1029 HBsAg positive chronic hepatitis B patients visited the National Hospital Organization (NHO) Nagasaki Medical Center. We included only patients who received more than 5 years of regular follow up and met the following criteria: (i) no comorbid HCC or signs of hepatic decompensation; (ii) no evidence of concurrent infection with hepatitis C virus, anti-HIV and/or hepatitis delta virus; and (iii) exclusion of other causes of chronic liver disease (alcoholism, hepatotoxic drugs, and autoimmune liver disease). A cohort of 392 patients was selected for further analysis. All participants were followed up every 3–6 months. The median follow-up duration was 14.0 years (range, 5–31) (Table 1). Serum samples were collected on a yearly basis and stored at $-20\,^{\circ}C$ until tested. LC was diagnosed by histological analysis and/or characteristic physical findings such as esophageal varices and splenomegaly. HCC was diagnosed by either histology or by typical imaging findings (arterial enhancement and venous washout by contrast-enhanced computed tomography

or magnetic resonance imaging). We received informed consent from each patient. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and the Ministry of Education, Culture, Sports Science and Technology of Japan, and was approved by the ethics committee of Nagasaki Medical Center.

Treatment

Two hundred and thirty-three patients received treatment for chronic hepatitis B. Of them, 33 patients were prescribed 30 mg of prednisolone daily for 3 weeks as corticosteroid withdrawal therapy (CWT). A total of 83 patients were administrated either IFN-α or -β. The regimen of IFN treatment was daily for 2 or 4 weeks, followed by 3 times a week for 2–20 weeks, with 3–6 MU as a single dose. The median duration of treatment was 4 weeks (range, 4–24). A total of 117 patients were prescribed nucleoside/nucleotide analogs (NA); 66 patients were treated with daily 100 mg of lamivudine (LAM). The median duration of LAM treatment was 81 months (range, 6.0–131). LAM-resistant mutation emerged in 37 (56%) out of 66 patients, and they were subsequently treated with an additional 10 mg adefovir dipivoxil. Fifty-one patients received daily 0.5 mg entecavir (ETV) as an initial therapy. The median duration of ETV treatment was 37 months (range, 3.0–77).

Serological testing

Serum hepatitis B e-antigen (HBeAg) and anti-HBe were tested using commercially available enzyme immunoassay kits (Abbott Japan, Tokyo, Japan). Quantitative measurement of HBsAg was performed using a HISCL HBsAg assay based on the chemiluminescence enzyme

Table 1 Clinical and laboratory findings in patients ($n = 392$)

Characteristic	Total ($n = 392$)	HBsAg seroclearance ($n = 50$)	HBsAg (+) ($n = 342$)	*P*-value
Age, years*	39.5 (9–74)	41.5 (23–59)	39.0 (9–74)	0.51
Male sex†	278 (71%)	41 (82%)	237 (69%)	0.04
HBeAg positive†	285 (73%)	29 (58%)	256 (75%)	0.05
HBV genotype, C/B	384/8	49/1	355/7	0.73
ALT, IU/L*	54 (8–2200)	35 (13–2200)	58 (8–1584)	0.03
HBsAg, log IU/mL*	3.6 (0.7–4.9)	3.3 (0.7–4.8)	3.7 (0.7–4.9)	0.02
HBV DNA, log copies/mL*	7.3 (ND–9.3)	5.9 (ND–8.8)	7.3 (1.7–9.3)	0.0005
With LC†	111 (28%)	22 (44%)	89 (26%)	0.01
CWT†	33 (8%)	5 (10%)	27 (8%)	0.78
IFN treatment†	83 (21%)	12 (24%)	71 (21%)	0.74
NA treatment†	117 (30%)	3 (6%)	114 (33%)	0.0001
Follow-up period, years*	14.0 (5–31)	17.5 (5–30)	13.0 (5–31)	0.0001

*Data are expressed as median (range).
†Data are expressed as number (%).
ALT, alanine aminotransferase; CI, confidence interval; CWT, corticosteroid withdrawal therapy; IFN, interferon; HBeAg, hepatitis B e-antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; LC, liver cirrhosis; NA, nucleoside/nucleotide analog.

immunoassay (Sysmex, Kobe, Japan). A positive linear correlation was observed between our method and the commonly used Architect HBsAg QT (Abbott Laboratories, Abbott Park, IL, USA). The assay had a quantitative range of −1.5 to 3.3 log IU/mL. The end titer was determined by diluting samples with normal human serum when initial results exceeded the upper limit of the assay range. Serum HBV DNA levels were determined using an AccuGene m-HBV Kit (Abbott Japan) with a linear quantitative range of 1.5–9.5 log copies/mL. Six HBV genotypes (A–F) were determined with the SMITEST HBV Genotyping Kit (MBL, Nagoya, Japan) by hybridization with type-specific probes immobilized on a solid-phase support.[15] HBsAg seroclearance was defined as two consecutive HBsAg levels of −1.5 log IU/mL at least 1 year apart. In 50 patients demonstrating HBsAg seroclearance, we quantified HBsAg levels in 637 samples in every year using stored serum samples. We collected ALT data every 3–6 months from medical records.

Statistical analysis

Data are expressed as median (range) or number (%). To compare characteristics between groups, the Mann–Whitney U-test was used for analysis of continuous variables and the χ^2-test was used for categorical variables. The Kaplan–Meier method was used to estimate the cumulative incidence of HBsAg seroclearance. Univariate and multivariate analyses were performed to identify factors associated with HBsAg seroclearance. Variables found to be significant in the univariate models were tested in a multivariate setting using the Cox proportional hazards regression models. All tests were performed using the IBM SPSS Statistics Desktop for Japan version 21.0 (IBM Japan, Tokyo, Japan). $P < 0.05$ was considered significant.

RESULTS

Baseline patient characteristics

THE CLINICAL AND laboratory findings of the 392 HBsAg positive carriers are shown in Table 1. The median age (range) at entry was 39.5 years (range, 9–74) and 278 (71%) were male. Two hundred and eighty-five patients (73%) were HBeAg positive. HBV genotype C was found in 384 patients (98%) and genotype B in eight patients. During the 14-year follow-up period, 50 patients demonstrated HBsAg seroclearance. The average annual incidence of HBsAg seroclearance was 0.91%. The following factors were associated with significant differences between patients with and without HBsAg seroclearance: male sex, ALT levels, presence of HBsAg and HBV DNA, comorbid LC and NA treatment.

Factors associated with HBsAg seroclearance

The cumulative incidences of HBsAg seroclearance were 5.4% at 10 years, 9.8% at 15 years and 23.5% at 20 years (Fig. 1). The median age of patients with HBsAg seroclearance was 56 years (range, 35–70). Forty-seven out of 275 untreated patients lost HBsAg, whereas three out of 117 NA-treated patients did so. In univariate analysis of baseline data, age of 40 years or more ($P = 0.02$), HBeAg negativity ($P = 0.001$), HBsAg of less than 3.3 log IU/mL ($P = 0.001$), HBV DNA of less than 6.0 log copies/mL ($P = 0.01$) and NA treatment ($P < 0.0001$) were significantly associated with HBsAg seroclearance (Table 2). In multivariate analysis, baseline HBsAg of less than 3.3 log IU/mL and NA treatment were independent predictive factors for HBsAg seroclearance (hazard ratio [HR], 2.22 [95% confidence interval [CI], 1.23–3.99], $P = 0.008$; and 0.12 [0.04–0.40], $P = 0.001$; respectively) (Table 2).

We made an additional analysis among the untreated cases separately from NA-treated cases. Among the 275 untreated patients, factors associated with HBsAg seroclearance in univariate analysis were age of 40 years or more ($P = 0.02$), HBeAg negativity ($P = 0.007$) and HBsAg of less than 3.3 log IU/mL ($P = 0.001$). Subsequent multivariate analysis confirmed that HBsAg of less than 3.3 log IU/mL was an independent predictive factor for HBsAg seroclearance (HR, 2.31 [1.29–4.14]; $P = 0.005$).
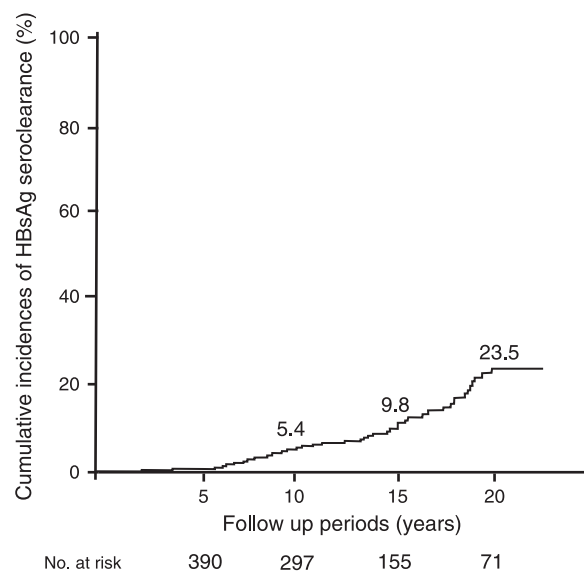

Figure 1 Cumulative incidences of hepatitis B surface antigen (HBsAg) seroclearance ($n = 392$). Cumulative incidences of HBsAg seroclearance in 392 HBsAg positive carriers were 5.4% at 10 years, 9.8% at 15 years and 23.5% at 20 years.

Table 2 Cox regression analysis of factors predicting hepatitis B surface antigen seroclearance (*n* = 392)

	Univariate analysis	*P*-value	Multivariate analysis	*P*-value
	Hazard ratio (95% CI)		Hazard ratio (95% CI)	
Age ≥40 years	1.97 (1.11–3.47)	0.02		
Male sex	1.83 (0.86–3.91)	0.12		
HBeAg negative	2.63 (1.48–4.65)	0.001		
ALT ≥50 IU/L	0.89 (0.50–1.60)	0.71		
HBsAg <3.3 log IU/mL	2.58 (1.48–4.50)	0.001	2.22 (1.23–3.99)	0.008
HBV DNA <6.0 log copies/mL	2.08 (1.19–3.14)	0.01		
With LC	1.00 (0.57–1.76)	0.99		
CWT	0.62 (0.24–1.58)	0.32		
IFN treatment	1.00 (0.52–1.93)	0.99		
NA treatment	0.11 (0.03–0.35)	<0.0001	0.12 (0.04–0.40)	0.001

ALT, alanine aminotransferase; CI, confidence interval; CWT, corticosteroid withdrawal therapy; IFN, interferon; HBeAg, hepatitis B e-antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; LC, liver cirrhosis; NA, nucleoside/nucleotide analog.

Among the 117 NA-treated patients, on the other hand, there was no significant factor associated with HBsAg seroclearance.

Kinetics of HBsAg levels in patients with HBsAg seroclearance

Figure 2 shows the kinetics of median HBsAg levels prior to HBsAg seroclearance. The median HBsAg titers at 20 and 10 years before HBsAg seroclearance, respectively, were 3.89 log IU/mL (7762 IU/mL) and 2.84 log IU/mL (692 IU/mL). The median HBsAg titers at 5, 3 and 1 year before HBsAg seroclearance were 1.84 log IU/mL (69.2 IU/mL), 0.78 log IU/mL (6.03 IU/mL) and −1.1 log IU/mL (0.08 IU/mL), respectively (Fig. 2a).

Clinical and virological characteristics associated with rapid versus slow decline of HBsAg levels

Hepatitis B surface antigen seroclearance was achieved approximately 5 years after HBsAg levels reached 2 log IU/mL (Fig. 2a). Accordingly, we tentatively categorized patients into two groups based on rapidity of seroclearance. The rapid decline group comprised patients who achieved HBsAg seroclearance within 5 years after confirmed HBsAg levels of 2 log IU/mL, and the slow decline group comprised those who achieved HBsAg seroclearance after 5 years. Figure 2 shows the kinetics of HBsAg in the rapid decline group (B) and slow decline group (C). Next, we compared the clinical characteristics between patients in both groups (Table 3). Levels of HBsAg and HBV DNA at baseline were significantly higher in the rapid decline group than the slow decline group. All patients were confirmed to be negative for HBeAg at HBsAg 2 log IU/mL

(data not shown). Compared with the slow decline group, the rapid decline group exhibited a significantly higher maximum ALT level (487 vs 146 IU/L [*P* = 0.03]) and lower prevalence of LC (32% vs 54% at baseline [*P* = 0.1], 36% vs 68% at HBsAg 2 log IU/mL [*P* = 0.03], 36% vs 68% at HBsAg seroclearance [*P* = 0.03]) during the follow-up period.

Relationship between HBsAg decline and ALT flares

Liaw *et al.* reported that an ALT elevation to 200 IU/L or fivefold the upper limit of normal is the minimum criterion of hepatic flare.[16,17] Such ALT threshold as flare has been widely accepted in the clinical studies since 1990s. Therefore, we defined an ALT flare as an ALT level of 200 IU/L or more, a year with ALT flare (flare [+]) as the occurrence of at least one ALT flare, and a year without ALT flare (flare [−]) as the absence of ALT flare in that year. Annual reductions of HBsAg were calculated by subtracting HBsAg levels of a given year from that of the previous year (e.g. A-B, B-C, C-D) (Fig. 3). Figure 4 shows the occurrence of ALT flares before HBsAg seroclearance in the rapid decline group (A) and the slow decline group (B). In the rapid decline group there were 47 flare (+) and 204 flare (−). In contrast, the slow decline group demonstrated 22 flare (+) and 362 flare (−) (Fig. 4a,b). Figure 4(c,d) compares the percentage of flare (+) between the rapid and slow decline groups before HBsAg seroclearance; the percentage of flare (+) is calculated by dividing the number of flare (+) by the total number of flare (+) and flare (−). From 10–20 years before HBsAg seroclearance, the percentage of flare (+) was significantly higher in the rapid decline group than the slow decline group (41.5% vs 8.1%, *P* < 0.001)
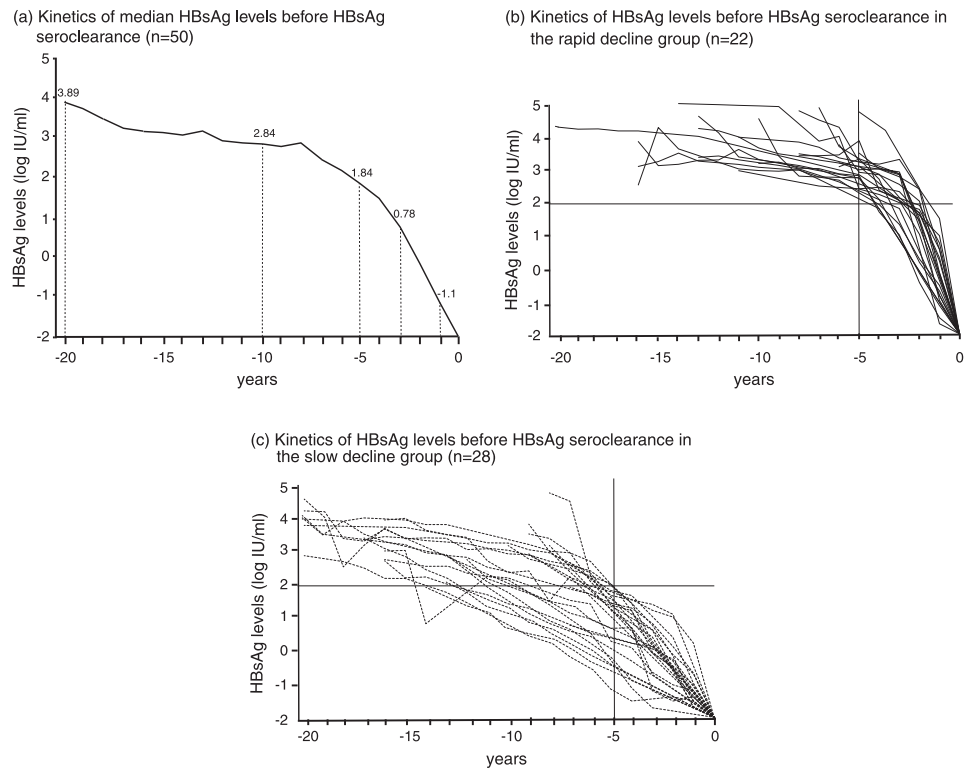
Figure 2 Kinetics of hepatitis B surface antigen (HBsAg) levels in the patients with HBsAg seroclearance (*n* = 50). (a) Kinetics of median HBsAg levels before HBsAg seroclearance in 50 patients. Median HBsAg titers were 3.89 and 2.84 log IU/mL at 20 and 10 years before HBsAg seroclearance, respectively. Median HBsAg titers were 1.84, 0.78 and −1.1 log IU/mL at 5, 3 and 1 year before HBsAg seroclearance, respectively. (b) Kinetics of HBsAg levels before HBsAg seroclearance in the rapid decline group. (c) Kinetics of HBsAg levels before HBsAg seroclearance in the slow decline group.

(Fig. 4c,d). From 5–10 years before HBsAg seroclearance, flare (+) was also more prevalent in the rapid decline group (21.6% vs 9.2%, *P* = 0.02) (Fig. 4c,d). The two groups did not differ in flare (+) percentage within 5% of HBsAg seroclearance (5.5% vs 0.7%, *P* = 0.06) (Fig. 4c,d).

Figure 4(e) summarizes the characteristics of hepatic flares in both groups. Namely, frequency, magnitude and the interval between the most recent flare and the point of 2 log IU/mL HBsAg levels. The frequency of flares before 2 log IU/mL of HBsAg level in the rapid decline group was significantly higher compared with that of the slow decline group (81.8% [18/22] vs 32.1% [9/28], *P* = 0.0005) (Fig. 4e, left). On the contrary, median ALT levels of the most recent flare before 2 log IU/mL HBsAg between the rapid and the slow decline group were not significantly different (396.5 IU/L [212–1063] vs 263.0 IU/L [208–2472]) (*P* = 0.18) (Fig. 4e, middle). Moreover, the median interval between the most recent flare and the point of 2 log IU/mL HBsAg was similar between the rapid (3.85 years [0–10.4]) and the slow (2.10 years [0–9.7]) decline group (*P* = 0.32) (Fig. 4e, right).

Comparison of median annual HBsAg reduction levels between flare (+) and flare (−) groups

Finally, we analyzed a total of 385 years with and without ALT flares (62 flare [+] and 323 flare [−]), from 5 to 20 years before HBsAg seroclearance, examining the relationship between annual HBsAg reduction and presence or absence of ALT flare. As shown in Figure 5, the median annual HBsAg reduction level was significantly greater in the flare (+) group than the flare (−) group (0.29 [−0.18 to 1.82] vs 0.17 log IU/mL per year [−0.34 to 0.77], *P* = 0.003). The same pattern was observed over the entire follow-up period, including 0–5 years before seroclearance.

DISCUSSION

THE PRESENT STUDY examined the long-term kinetics of reductions of serum HBsAg levels in patients with chronic HBV infection and confirmed HBsAg seroclearance. Serum HBsAg levels are a surrogate marker

Table 3 Comparison of clinical characteristics between patients in the rapid and slow decline groups ($n = 50$)

Characteristic	Rapid decline group ($n = 22$)	Slow decline group ($n = 28$)	*P*-value
At baseline			
Age, years*	42 (23–57)	41 (23–59)	0.95
Male gender†	18 (82%)	23 (82%)	0.73
HBeAg positive †	14 (64%)	15 (54%)	0.67
HBV genotype, C/B	21/1	28/0	0.90
ALT, IU/L*	69 (13–2200)	26 (14–440)	0.1
HBsAg, log IU/mL*	3.5 (2.4–4.8)	3.1 (0.7–4.6)	0.02
HBV DNA, log copies/mL*	6.6 (2.8–8.4)	4.5 (ND–8.8)	0.01
With LC†	7 (32%)	15 (54%)	0.1
During follow up			
Maximum ALT, IU/L*	487 (28–2200)	146 (17–2472)	0.03
CWT†	1 (4.5)	4 (14)	0.37
IFN treatment†	5 (23)	7 (25)	0.85
NA treatment†	3 (14)	0 (0)	0.08
At HBsAg 2 log IU/mL			
Age, years*	52 (32–64)	45 (28–63)	0.36
ALT, IU/L*	25 (11–108)	27 (14–96)	0.45
Platelets, $\times 10^4$/mL*	16.8 (9.0–30.7)	16.7 (7.6–29.4)	0.87
AFP, ng/mL*	3.0 (1.0–4.0)	3.0 (1.0–10.0)	0.58
HBV DNA, log copies/mL*	3.0 (ND–6.1)	3.4 (ND–6.5)	0.88
With LC†	8 (36%)	19 (68%)	0.03
At HBsAg seroclearance			
Age, years*	56 (35–68)	53 (35–70)	0.38
With LC†	8 (36%)	19 (68%)	0.03
After HBsAg seroclearance			
Occurrence of HCC†	0 (0%)	2 (7.1%)	1.00

*Data are expressed as median (range).
†Data are expressed as number (%).
AFP, α-fetoprotein; ALT, alanine aminotransferase; CI, confidence interval; CWT, corticosteroid withdrawal therapy; IFN, interferon; HCC, hepatocellular carcinoma; HBeAg, hepatitis B e-antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; LC, liver cirrhosis; NA, nucleoside/nucleotide analog.

for intrahepatic, transcriptionally active cccDNA, and a reduction of HBsAg levels may indicate a reduction of intrahepatic cccDNA and induction of immune control over HBV.[18,19] Several recent studies reported that lower baseline HBsAg levels and greater annual reductions of HBsAg levels were independent predictive factors for subsequent HBsAg seroclearance.[13,14,20,21] Multivariate analysis in our present study confirmed the lower levels of baseline HBsAg as a positive predictive factor for HBsAg seroclearance. Surprisingly, NA treatment was revealed to be a negative predictive factor. The negative association was validated by the observation in which inactive carriers showed larger decline of HBsAg compared with patients treated with either IFN or NA (data not shown). Kim *et al.* reported HBsAg seroclearance after NA therapy in a large scale retrospective study. During median follow-up period

of 6 years, the annual seroclearance rate was 0.33%,[22] being comparable with that of the untreated cohort (0.5–2.26%).[7–9] Our findings that NA treatment was negatively associated with HBsAg loss could have been influenced by unidentified confounders, and we speculate at least that NA therapy does not confer an additional therapeutic benefit for either decline or seroclearance of HBsAg in chronic HBV carriers.

Previous studies in Europe and the USA reported that IFN treatment could induce HBsAg seroclearance.[23–26] Nevertheless, in our study, IFN treatment was not a significant predictive factor for HBsAg seroclearance. This discrepancy may be caused by differences in genetic factors, HBV genotypes and durations of IFN treatment (in the past, almost all patients in Japan were treated for 4 or 8 weeks).
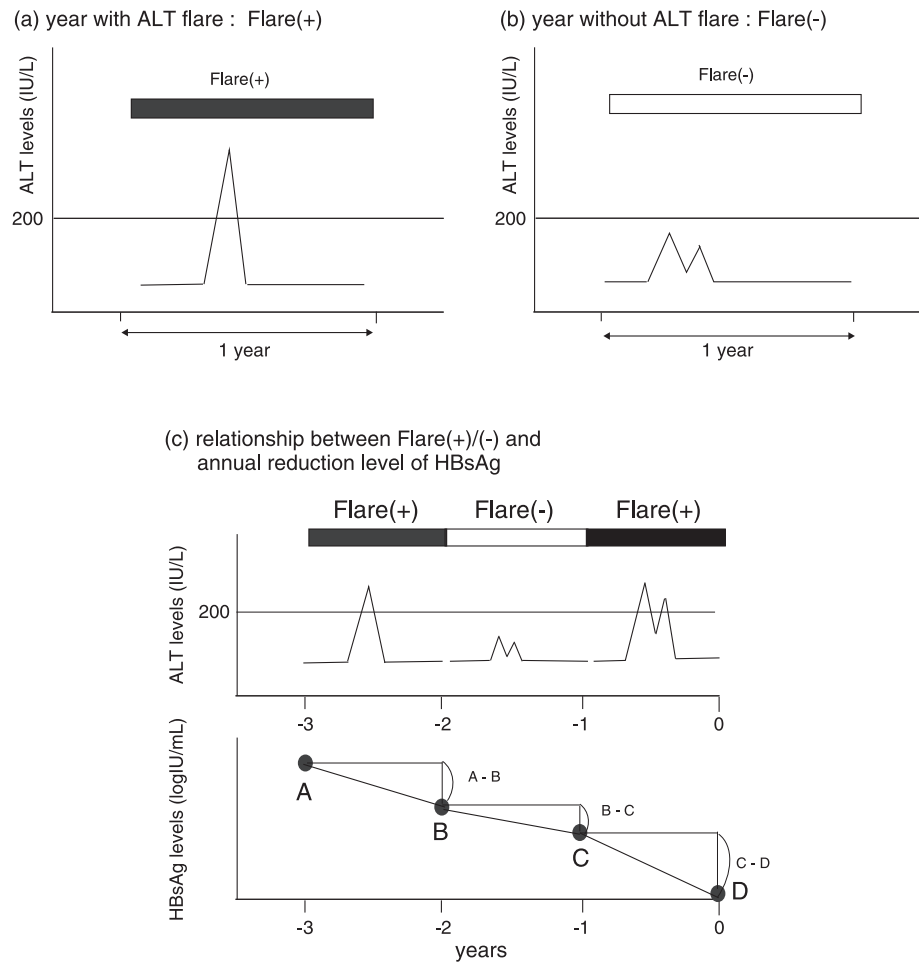
Figure 3 Definition of year with alanine aminotransferase (ALT) flare: flare (+), year without ALT flare: flare (−) and relationship between flare (+)/(−) and annual reduction in hepatitis B surface antigen (HBsAg) level. (a) A year with ALT flare (flare [+]) was defined by the occurrence of at least one ALT flare (ALT ≥200 IU/L). Filled rectangle indicates flare (+). (b) A year without ALT flare (flare [−]) was defined by the absence of ALT flare. Open rectangle indicates flare (−). (c) Annual reductions in HBsAg levels were calculated by subtracting HBsAg levels of a given year from that of the previous year (e.g. A-B). Annual HBsAg reductions in flare (+) correspond to HBsAg levels from A-B and C-D, while those in flare (−) correspond to those from B-C.

Based on long-term serial HBsAg data, we estimated that median HBsAg levels were 2.84 log IU/mL at 10 years prior to HBsAg seroclearance. Specifically, HBsAg levels were 1.84 log IU/mL and 0.78 log IU/mL at 5 years and 3 years before HBsAg seroclearance, respectively (Fig. 2). Arai *et al.* demonstrated that HBsAg seroclearance in HBV carriers with HBsAg levels of less than 3 log IU/mL occurred at a significantly higher rate than in those with higher HBsAg levels (log–rank test, $P < 0.01$).[21] Tseng *et al.* reported that HBsAg levels of less than 2 log IU/mL predicted HBsAg loss within 6 years with a diagnostic accuracy of 91.5%, sensitivity of 83.3% and specificity of 92.1% in HBeAg seroconverters.[20] We speculate that

lower HBsAg levels may accelerate HBsAg decrease and seroclearance.

The incidence of HCC after HBsAg seroclearance has been reported to be 1.3–20% in Asian studies.[4,5,27] In this study, HCC occurred in two patients (4%) after HBsAg seroclearance (Table 3). In addition, there were eight cases of HCC (16%) in terms of the incidence of HCC before and after HBsAg seroclearance (−5 to ≥8 years). Comorbid LC occurred in six cases (75%). The mean age of HCC development was 57.0 ± 7.8 years. Previous studies showed that development of HCC after HBsAg seroclearance was associated with comorbid LC and an age of greater than 50 years at the time of HBsAg seroclearance.[2,3,5,6,27] HBsAg
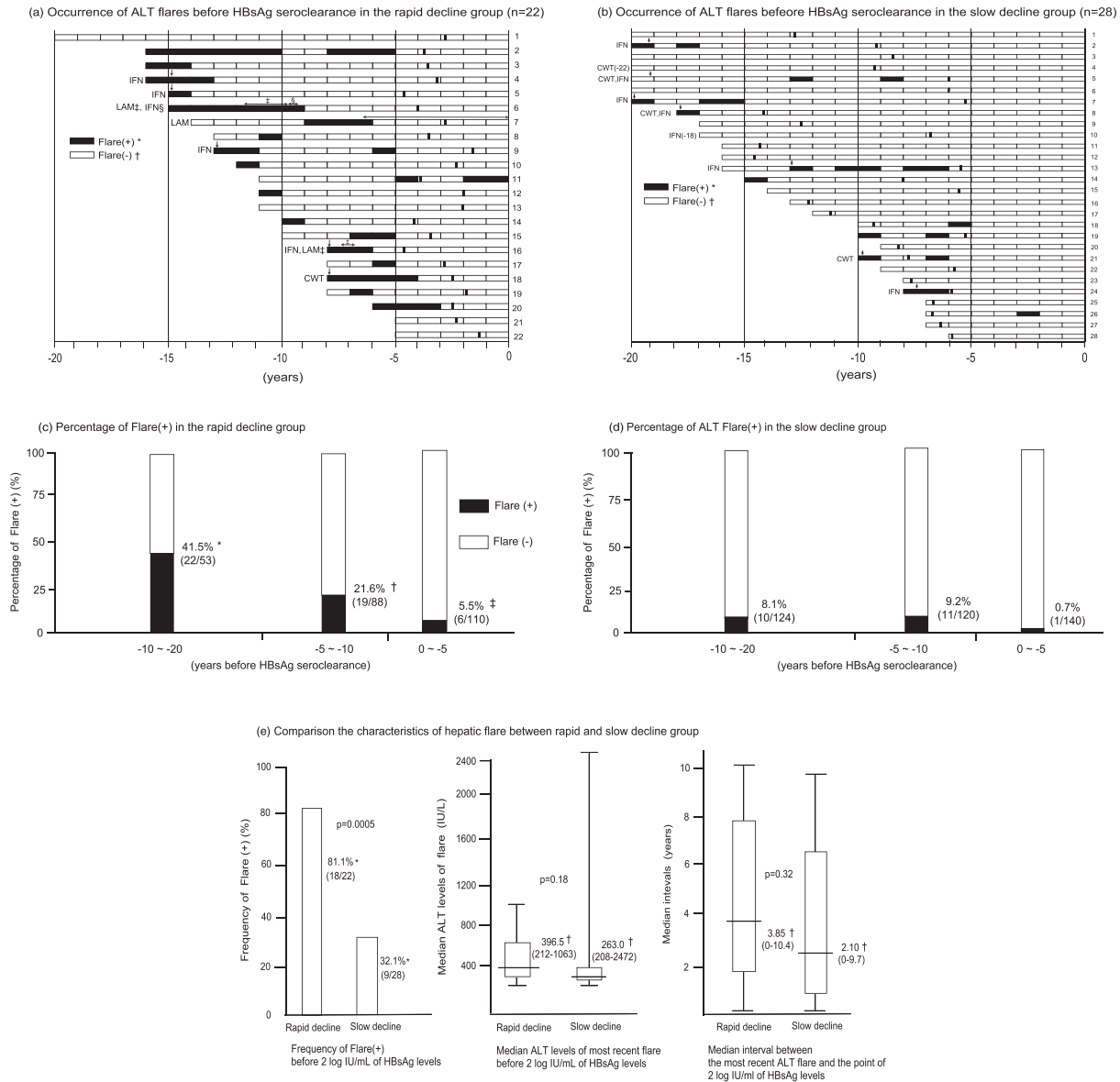
Figure 4 Occurrence of alanine aminotransferase (ALT) flares in the patients with HBsAg seroclearance and comparison of percentage of flare (+) between rapid and slow decline group (*n* = 50). (a) Rapid decline group demonstrates a total of 47 flare (+) and 204 flare (−). Arrows indicate initiation or duration of therapy. ‡LAM (lamivudine), §IFN (interferon). Thick lines indicate the points of 2 log IU/mL of HBsAg levels. (b) Slow decline group shows 22 flare (+) and 362 flare (−). *Filled rectangles indicate flare (+). †Open rectangles indicate flare (−). Arrows indicate initiation of therapy. Figure in parentheses indicate mean year of initiation of therapy. Thick lines indicate the points of 2 log IU/mL of HBsAg levels. (c) Percentage of flare (+) in the rapid decline group. (d) That in the slow decline group. Data are expressed as % (the number of flare [+] divided by total number of flare [+] and flare [−]). *†Significantly different compared with percentage of flare (+) in slow decline of HBsAg. *$P < 0.001$, †$P = 0.02$, ‡$P = 0.06$. (e) *Data are expressed as % (the number of Flare [+] divided by the total number of patients). †Data are expressed as median (range).

seroclearance is associated with favorable long-term outcomes in chronic HBV carriers. However, patients with established LC should undergo ongoing surveillance for HCC.

In the present study, HBsAg kinetics in patients with HBsAg seroclearance suggested that patterns of HBsAg decline were divided into the rapid and slow decline groups. The rapid decline group demonstrated a significantly
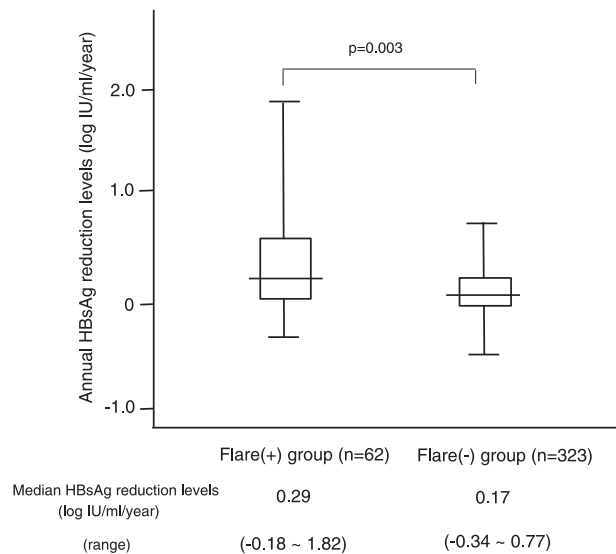
Figure 5 Comparison of median annual reduction levels between flare (+) and flare (−) groups in more than 5 years before hepatitis B surface antigen (HBsAg) seroclearance. The annual reductions in HBsAg levels, calculated as defined in Fig. 3, for 5–20 years before HBsAg seroclearance. Positive numbers represent reductions in HBsAg levels.

higher maximum ALT level during the follow-up period and a lower rate of progression to LC.

Previous studies reported that ALT level of more than 200 IU/L was associated with HBsAg seroclearance.[22,28] Higher ALT levels reflect robust host immune response to HBV, resulting in anti-HBe seroconversion and HBV DNA reduction. On the other hand, HBsAg level of 2 log IU/mL has been shown as a strong predictive factor for HBsAg seroclearance.[20,29] Regarding types of ALT flare, Flink *et al.* reported that host-induced flare was an independent predictor of response to PEG IFN in chronic hepatitis B.[30] Host-induced flare was defined as flare followed by a decrease in HBV DNA. In contrast, virus-induced flare, which occurs after an increase in HBV DNA and indicates increased expression of viral antigen, was not associated with positive treatment response. Sonneveld *et al.* reported that host-induced flare resulted in pronounced declines in HBsAg levels, and patients who achieved a decline in HBsAg of more than 0.5 log IU/mL within 4 weeks after the flare cleared HBsAg in 64% (7/11) of cases.[31] In this study, we focused mainly on the frequency in flare (+) in the rapid and the slow decline group. Flare (+) was more frequent in the rapid decline group than the slow decline group (Fig. 4). Moreover, serum HBsAg levels were reduced more significantly in the flare (+) group than the flare (−) group (Fig. 5). Regarding the recent ALT

fluctuation before HBsAg loss, we made an additional analysis between the rapid and the slow decline groups for: (i) ALT levels within 5 years prior to HBsAg loss; and (ii) those during the phase of HBsAg levels below 2 log IU/mL. The median ALT levels within 5 years prior to HBsAg loss were revealed to be within normal limit (24 vs 27 IU/L, $P = 0.23$). Also, those during the phase of HBsAg levels below 2 log IU/mL were found to be of similar level (22 vs 28 IU/L, $P = 0.05$). We could not detect significant ALT elevations at the time of close to HBsAg seroclearance, with some exceptions (no. 11 in the rapid group, no. 26 in the slow group; Fig. 4a,b).

Our study could not identify flare types, but we attribute our results to ALT flare being the host immune response to HBV-infected hepatocytes. The fluctuating but sustained nature of host immune response within certain periods, represented by the number and the density of hepatic flares, is associated with rapid HBsAg seroclearance in the near future, independently with the magnitude and the proximity of flare. The biological plausibility of these findings is still elusive, but robust inflammation may cause sustained epigenetic repression of HBV transcription, as reported for the long-term effect of IFN-α.[32] Wong *et al.* reported that hepatic flare was associated with profound HBsAg decline during 2 years of NA therapy for HBeAg negative patients.[33] Collectively, we confirmed that it was not the recent (within 5 years before) ALT fluctuation before HBsAg loss but the past ALT flare (>10 years before), being involved in HBsAg decline at that time, affected the following phase of rapid HBsAg reduction or seroclearance

The mechanism underlying the lower prevalence of LC observed in the rapid decline group is still not clear, but integration of HBV into patients with LC is probably associated with a slow decline of HBsAg. Further studies are required to demonstrate this point.

Although our study included long-term follow-up data of patients with HBsAg seroclearance, it had some limitations. First, it was a single-center retrospective study with a small sample size. Second, most of our patients were infected with HBV genotype C. A recent study reported distinct kinetics of HBsAg during PEG IFN, according to HBV genotype.[34] Third, we investigated serial HBsAg values only in HBsAg seroclearance cases. Impact of ALT flares on the reduction of HBsAg levels in patients with persistent HBsAg is still elusive. Finally, our results need to be validated by further studies investigating large study populations and different HBV genotypes.

In summary, in this study we demonstrated: (i) independent predictive factors for HBsAg seroclearance by multivariate analysis; (ii) the long-term kinetics of serum HBsAg levels in patients with HBsAg seroclearance; and

(iii) the impact of hepatic flare on the decline in HBsAg levels. The independent predictive factors for HBsAg seroclearance were lower levels of HBsAg ($<3.3 \log$ IU/mL) and NA therapy. HBsAg levels contributed more significantly to HBsAg seroclearance than HBV DNA levels. The long-term kinetics of HBsAg levels revealed that the median HBsAg levels 10 and 5 years before seroclearance were approximately 3 and 2 log IU/mL, respectively. Our findings may help to estimate the remaining years toward HBsAg seroclearance in patients with chronic hepatitis B. ALT flare reflects destruction of HBV-infected hepatocytes and leads to reduction of intrahepatic cccDNA. As a result, serum HBsAg levels decrease and time until seroclearance is reduced. Transient hepatic flares without disease progression during immunomodulatory therapy, for instance, with IFN-based treatments or with a novel oral agonist of Toll-like receptor-7,[35] may play an important role in achieving more frequent HBsAg seroclearance after treatment for chronic hepatitis B.

We conclude that hepatic flares promote rapid declines and greater annual reductions of serum HBsAg levels in patients with HBsAg seroclearance.

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