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Figure captions

Figure 1. Study design.

To properly determine the effect of Vitamin D, patients who became HCV RNA negative at week 4 were excluded. The remaining patients were stratified according to viral load (decline in HCV RNA level at week 4 <1 log IU/ml or ≥ 1 log IU/ml) and randomly assigned to a vitamin D or control group. The patients randomized to the vitamin D group received 1,000 IU /day vitamin D, beginning at week 8 and continuing until the end of treatment.

Figure 2. Enrollment, randomization or assignment, and follow-up of study patients

Forty-two patients were randomized to the vitamin D group and 42 to the control group.

Figure 3. HCV RNA disappearance rate at week 24 in the vitamin D and control groups.

All patients were treated with PEG-IFN plus RBV, with the vitamin D group also receiving vitamin D. IL28B TT: rs8099917 genotype TT patients, who were sensitive to IFN treatment

Figure 4. Differences in HCV RNA disappearance rate at week 24 stratified by IL28B genotype.

VR rates at week 24 were assessed in patients with the IL28B SNP rs8099917 TT and TG/GG genotypes randomized to the vitamin D and control groups. Vitamin D(-): control group, treated with PEG-IFN/RBV. Vitamin D(+): vitamin D group, treated with PEG-IFN/RBV plus vitamin D.

IL28b TT: IL28B SNP rs8099917 genotype TT, which is sensitive to IFN therapy. IL28B TG/GG:

IL28B SNP rs8099917 genotypes TG/GG, which are relatively resistant to IFN therapy.

Figure 5. Decrease in serum viral load from weeks 8 to 24 in patients with the TG/GG

genotypes randomized to the vitamin D and control groups. The decrease in viral load was

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significantly greater in the vitamin D than in the control group.

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Table 1. Baseline Characteristics of patients in the control and vitamin D groups.

Characteristic		Control Group n=42	Vitamin D group n=42	P value
Age	years	59 (36-70)	60 (30-78)	0.59
Male:Female	ratio	23:19	20:22	0.58
Previous IFN* therapy	No/yes	28 / 9	32 / 9	1
Body mass index	Kg/m ²	22.5 (18.9-31.6)	22.1 (18.1-27.7)	0.43
Stage of fibrosis	0-2/3,4/ND	19 / 17 / 5	17 / 12 / 13	0.15
IL28B rs8099917	TT/TG/GG/ND	30 / 8 / 0 / 2	29 / 8 / 2 / 3	0.89
HCV-RNA log ₁₀	IU/ml	6.3 (4.7-7.3)	6.3 (4.7-7.3)	1
HCV genotype 1b	No.(%)	41(100%)	42(100%)	
Platelet count	no./μl	18.3 (8.3-36.2)	15.2 (7.8-27.2)	<u>0.01</u>
Hemoglobin	g/dl	14.5 (10.9-16.4)	13.7 (11.7-17.0)	0.15
ALT*	IU/L	60.0 (10.0-495.0)	51.0 (14.0-250.0)	0.34
AST*	IU/L	51.0 (12.0-236.0)	44.5 (16.0-167.0)	0.31
γ-GTP*	IU/L	15.5 (13.0-282.0)	30.0 (11.0-240.0)	0.10
HbA1c	%	5.1 (3.9-10.2)	5.1 (4.4-11.8)	0.47
T-Cho*	mg/dl	173.0 (125.0-242.0)	180.0 (113.0-257.0)	0.37
25(OH)vitamin D3	ng/ml	25.0 (14.0-40.0)	22.0 (12.0-29.0)	<u>0.028</u>

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*ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ -GTP, γ -glutamyl
transpeptidase; HbA1c, hemoglobin A1c; T-Cho, total cholesterol

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Table 2. Baseline characteristics of the study patients based on HCV RNA detection at 24

weeks

Characteristic		HCV RNA(-) n=56	HCV RNA(+) n=28	P value
Age	years	61.0 (30.0-78.0)	58.5 (36.0-71.0)	0.75
Male:Female	ratio	29:27	14:14	1
Previous IFN therapy	no	11	7	0.88
	yes	40	20	
Body mass index	Kg/m ²	22.2 (18.1-30.8)	22.4 (19.0-31.6)	0.67
Stage of fibrosis	0-2/3,4/ND	25 / 16 / 15	10 / 13 / 5	0.35
IL28B rs8099917	TT/TG/GG/ND	45 / 6 / 1 / 4	14 / 10 / 1 / 3	<u>0.003</u>
HCV-RNA log ₁₀	IU/ml	6.3 (4.7-7.3)	6.3 (4.7-7.3)	1
HCV genotype 1b	No.(%)	56(66.7%)	28(33.3%)	
Platelet count	no./μl	16.7 (9.0-27.2)	15.7 (7.8-36.2)	0.78
Hemoglobin	g/dl	14.3 (11.6-16.3)	13.7 (10.9-17.0)	0.71
ALT*	IU/L	50.5 (12.0-495.0)	64.0 (10.0-246.0)	0.14
AST*	IU/L	51.0 (13.0-182.0)	44.5 (12.0-236.0)	0.094
γ-GTP*	IU/L	35.0 (11.0-282.0)	61.0 (14.0-240.0)	0.057
HbA1c*	%	5.1 (4.6-11.8)	5.1 (3.9-10.2)	0.51
T-Cho*	mg/dl	179.5 (125.0-257.0)	173.0 (116.0-242.0)	0.49

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25(OH)vitamin D ₃	ng/ml	24.0 (12.0-40.0)	20.0 (12.0-39.0)	0.29
Vitamin D +/-	%	33 / 23	9 / 18	<u>0.029</u>
Reduction case	-/PEG/RBV/+	20 / 4 / 19 / 8	11 / 0 / 7 / 8-	
Adherence to PEG IFN	%	69.0%	No case	
Adherence to RBV	%	82.5%	80.8%	0.76
Adherence to both	PEG IFN (%)	83.0%	81.7%	0.66
PEGIFN+RBV	RBV (%)	79.6%	81.7%	0.71

*ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ -GTP, γ -glutamyl

transpeptidase; HbA1c, hemoglobin A1c; T-Chol, total cholesterol

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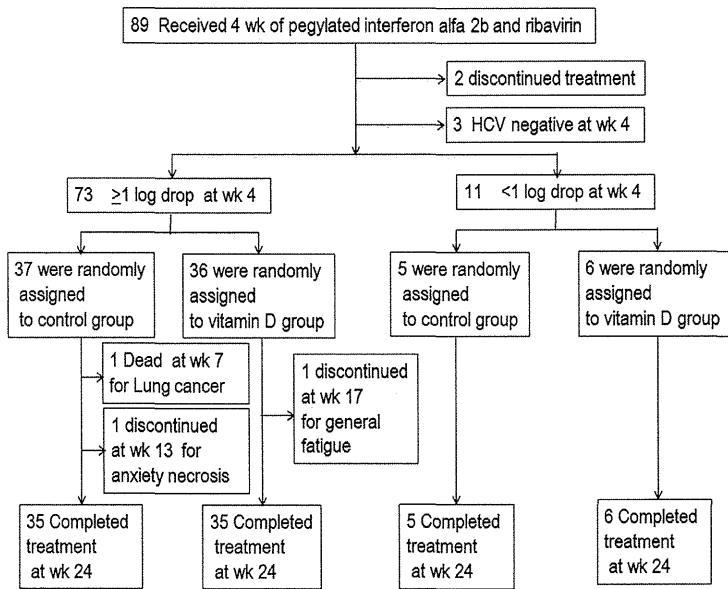
Table 3. Logistic regression analysis of factors contributing to undetectable HCV RNA at week 24.

	Crude OR (95%CI)	P value	Adjusted OR (95%CI)	P value
Age	1.00 (0.96-1.05)	0.81		
Gender	1.29 (0.48-3.46)	0.50		
Platelet count	1 (0.91-1.1)	0.79		
ALT*	0.99 (0.99-1.00)	0.051		
AST*	0.99 (0.98-1.0)	0.054		
γ -GTP*	0.99 (0.98-1)	0.42		
Virus amount	1.03 (0.43-2.46)	0.67		
Previous IFN therapy	2.54 (0.93-6.98)	0.24		
Vitamin D +/-	2.54 (0.93-6.98)	0.062	3.12 (1.03-9.5)	<u>0.04</u>
IL28B	4.96 (1.59-15.41)	0.004	5.85 (1.75-19.57)	<u>0.004</u>

*ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ -GTP, γ -glutamyl transpeptidase

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Figure. 2

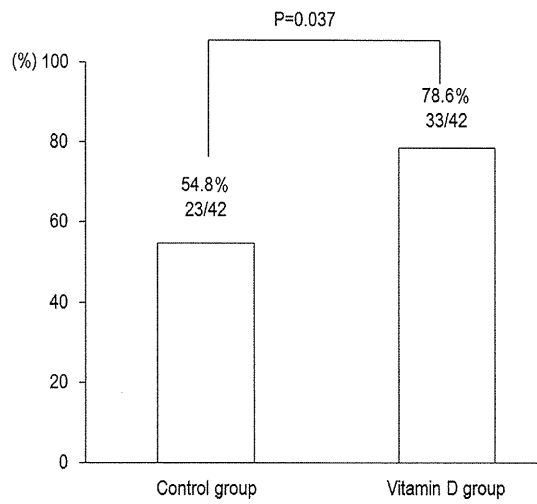


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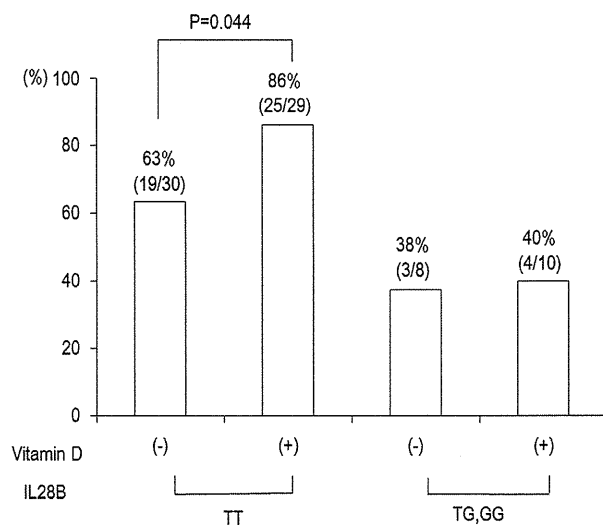
Figure. 3



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Figure. 4

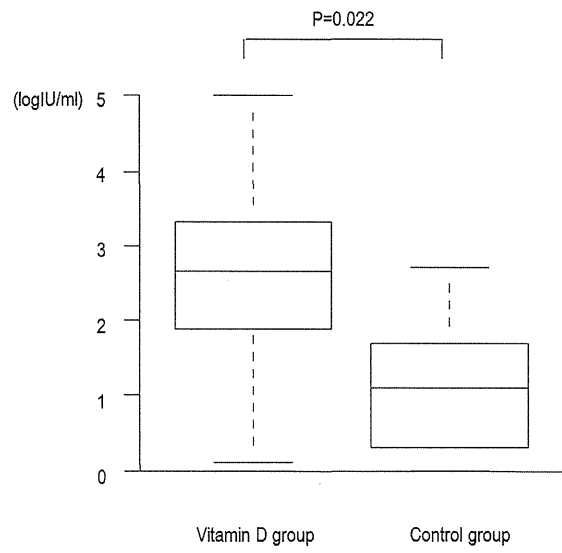


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Figure. 5



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Risk Factors for Long-Term Persistence of Serum Hepatitis B Surface Antigen Following Acute Hepatitis B Virus Infection in Japanese Adults

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The proportion of patients who progress to chronicity following acute hepatitis B (AHB) varies widely worldwide. Moreover, the association between viral persistence after AHB and hepatitis B virus (HBV) genotypes in adults remains unclear. A nationwide multicenter study was conducted throughout Japan to evaluate the influence of clinical and virological factors on chronic outcomes in patients with AHB. For comparing factors between AHB patients with viral persistence and those with self-limited infection, 212 AHB patients without human immunodeficiency virus (HIV) coinfection were observed in 38 liver centers until serum hepatitis B surface antigen (HBsAg) disappeared or a minimum of 6 months in cases where HBsAg persisted. The time to disappearance of HBsAg was significantly longer for genotype A patients than that of patients infected with non-A genotypes. When chronicity was defined as the persistence of HBsAg positivity for more than 6 or 12 months, the rate of progression to chronicity was higher in patients with genotype A, although many cases caused by genotype A were prolonged cases of AHB, rather than chronic infection. Multivariate logistic regression analysis revealed only genotype A was independently associated with viral persistence following AHB. A higher peak level of HBV DNA and a lower peak of alanine aminotransferase (ALT) levels were characteristics of AHB caused by genotype A. Treatment with nucleotide analogs (NAs) did not prevent progression to chronic infection following AHB overall. Subanalysis suggested early NA initiation may enhance the viral clearance. **Conclusion:** Genotype A was an independent risk factor for progression to chronic infection following AHB. Our data will be useful in elucidating the association between viral persistence after AHB, host genetic factors, and treatment with NAs in future studies. (HEPATOLOGY 2014;59:89-97)

Abbreviations: AHB, acute hepatitis B; ALT, alanine aminotransferase; anti-HBc, antibody to hepatitis B core antigen; anti-HBs, antibody to HBsAg; HBeAg, hepatitis B e-antigen; CLIA, chemiluminescent enzyme immunoassay; ELA, enzyme immunoassay; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HIV, human immunodeficiency virus; IgM, immunoglobulin M; anti-HBe, antibody to HBeAg; NAs, nucleotide analogs; RPHA, reverse passive hemagglutination.

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Received January 5, 2013; accepted July 10, 2013.

Hepatitis B virus (HBV) infection is one of the most common persistent viral infections in humans. Approximately 2 billion people worldwide have been exposed to HBV and 350 million of them remain persistently infected.¹ The incidence of HBV infection and the patterns of transmission vary greatly worldwide among different population subgroups.² In Western countries, chronic HBV infection is relatively rare and acquired primarily in adulthood by way of sexual transmission or the use of injectable drugs. Meanwhile, in Asia and most of Africa, the majority of infections are the result of transmission from an infected mother to her newborn. However, very few studies of acute hepatitis B (AHB) in adults have been reported.

HBV genomic sequences vary worldwide and have been classified into at least eight genotypes (A through H) based on an intergroup divergence of 8% or more over the complete nucleotide sequence.^{3,4} These genotypes have distinct geographic distributions.⁵⁻⁷ In particular, genotype A is predominant in Northwestern Europe, the United States, Central Africa, and India.⁸ The Japanese have been infected with genotypes B and C since prehistoric times.¹⁰ Recently, many lines of evidence have revealed among the Japanese an increase in acute infection with HBV genotype A following sexual transmission.^{11,12} As a result of this increasing transmission of genotype A, the distribution of HBV genotypes in Japan clearly differs among patients with acute and chronic infections.¹³ Moreover, recent studies suggest that acute infection with HBV genotype A may be associated with an increased risk of progression to persistent infection.¹⁵ Indeed, the prevalence of HBV genotype A in chronic hepatitis B patients doubled in Japan between 2000-2001 and 2005-2006 (1.7% versus 3.5%).¹¹

Human immunodeficiency virus (HIV)-1 infection results in an immunodeficient state, with the virus sharing routes of transmission with HBV. HIV-related immunodepletion influences the natural history of HBV infection, and epidemiological studies have

revealed that HIV-positive patients are more likely to have a prolonged acute illness following HBV infection and lower rates of hepatitis B e-antigen (HBeAg) clearance.¹⁶ Therefore, in this study patients with coinfection of HIV were excluded to examine the influence of HBV genotype directly without the confounding influence of HIV.

From 2005 to 2010, a multicenter cohort study was conducted throughout Japan on 212 patients with AHB. The aim of this cohort study was to assess the influence of clinical and virological factors, including HBV genotypes and treatment with nucleotide analogs (NAs), on AHB patients who became persistently infected.

Patients and Methods

Patients With AHB. The multiple-source cohort included 212 randomly selected AHB patients without coinfection of HIV. From 2005 through 2010, the study participants were recruited from 38 liver centers throughout Japan. The cohort included patients who were admitted to the hospital because of AHB and who visited the hospital every month after being discharged. The diagnosis of AHB was contingent on the rapid onset of clinical symptoms accompanied by elevated serum alanine aminotransferase (ALT) levels, the detection of serum hepatitis B surface antigen (HBsAg), and a high-titer antibody to hepatitis B core antigen (anti-HBc) of the immunoglobulin M (IgM) class. Patients with initial high-titer anti-HBc (>10.0 S/CO) were diagnosed as having an exacerbation of chronic hepatitis B and were excluded. If the patient had been tested previously, the absence of serum HBsAg and anti-HBc before admission was verified from the medical record to discriminate a new infection from an acute exacerbation of a persistent infection. Patients with acute hepatitis A, hepatitis C, and drug- or alcohol-induced acute hepatitis were also excluded; hepatitis D virus infection was not determined because of its extreme rarity in Japan. The study protocol conformed to the 1975 Declaration of

Supported by Japanese Ministry of Health, Labor and Welfare grant H21-003 and Japanese Ministry of Education, Culture, Sports, Science and Technology grant 22790679.

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DOI 10.1002/hep.26635

Potential conflict of interest: Nothing to report.

Additional Supporting Information may be found in the online version of this article.

Table 1. Characteristics of Patients With Genotype A or a Non-A Genotype Acutely Infected With Hepatitis B Virus

Features	Genotype A (n = 107)	Non-A Genotypes (n = 105)*	P Value
Age (years)	36.3 ± 12.0	40.7 ± 14.3	0.032
Male sex	102 (95.3)	75 (71.4)	<0.001
HBeAg positive	104 (97.2)	79 (75.2)	<0.001
ALT (IU/L)	1210 ± 646	2225 ± 2851	0.045
Total bilirubin (mg/dL)	9.9 ± 9.4	7.5 ± 6.7	0.115
HBV DNA (log copies/mL)	7.0 ± 1.5	5.8 ± 1.5	<0.0001
Duration until disappearance of HBsAg (month)	6.7 ± 8.5	3.4 ± 6.5	<0.0001
Persistence of HBsAg positivity more than 6 months	25 (23.4)	9 (8.6)	0.003
Persistence of HBsAg positivity more than 12 months	8 (7.5)	1 [†] (0.9)	0.018
Sexual transmission	81/84 (96.4) [‡]	71/79 (89.9) [§]	0.095
Treatment with NAs	61 (57.0)	42 (40.0)	0.013

Data are presented as n (%), mean ± standard deviation. HBV, hepatitis B virus; HBeAg, hepatitis B e-antigen; ALT, alanine aminotransferase; NAs, nucleotide analogs.

*Non-A genotypes include genotypes B, C, D, F and H (n = 25, 77, 1, 1, and 1, respectively).

[†]One patient had genotype C.

[‡]Transmission routes were unknown for 23 patients.

[§]Transmission routes were unknown for 26 patients.

Helsinki and was approved by the Ethics Committees of the institutions involved. Every patient gave informed consent for this study.

Serological Markers of HBV Infection. HBsAg, HBeAg, antibodies to HBsAg (anti-HBs), HBeAg (anti-HBe), and HBcAg, and anti-HBc of the IgM class were tested by a chemiluminescent enzyme immunoassay (CLIA) by ARCHITECT (Abbott Japan, Tokyo, Japan). HBV DNA measurements were performed using a real-time polymerase chain reaction (PCR) assay (Cobas TaqMan HBV Auto; Roche Diagnostics, Tokyo, Japan).

Genotyping of HBV. The six major HBV genotypes (A through F) were determined serologically by enzyme immunoassay (EIA) using commercial kits (HBV GENOTYPE EIA; Institute of Immunology, Tokyo, Japan). This method is based on the pattern of detection by monoclonal antibodies of a combination of epitopes on preS2-region products, which is specific for each genotype.^{17,18} Samples for which EIA could not determine the genotype were examined by direct sequencing of the pre-S2/S gene, followed by phylogenetic analysis.

Treatment With NAs. Treatments with NAs were performed using lamivudine or entecavir for more than 3 months. The individual clinicians determined if NAs were administered to patients, and when the treatment was to be started. The time to onset of treatment with NAs was measured in days from onset of AHB.

Statistical Analysis. Categorical variables were compared between groups by the chi-squared test and noncategorical variables by the Mann-Whitney *U* test.

A *P* value less than 0.05 was considered significant. Multivariate analysis was performed using a backward stepwise logistic regression model to determine independent factors for viral persistence following AHB. Variables in the multivariate analysis were selected based on variables that were marginally significant with *P* < 0.1 in univariate analysis. Maintenance of HBsAg positivity was analyzed using the Kaplan-Meier method and significance was tested with the log-rank test. STATA Software (StataCorp, College Station, TX) v. 11.0 was used for analyses.

Results

Comparison of Characteristics Between Genotype A and Non-A Genotype AHB Patients. A total of 107 AHB patients (50.5%) were infected with genotype A while 105 AHB patients (49.5%) were infected with non-A genotypes, including genotypes B (25 [11.8%]), C (76 [35.8%]), D (1 [0.5%]), F (1 [0.5%]), and H (1 [0.5%]). Compared to those infected with non-A genotypes, genotype A patients were significantly younger (36.3 ± 12.0 versus 40.7 ± 14.3 years, *P* = 0.032), predominantly men (95.3% versus 71.4%, *P* < 0.001), and more frequently positive for HBeAg (97.2% versus 75.2%, *P* < 0.001). Moreover, genotype A patients had a lower peak ALT levels (1,210 ± 646 versus 2,225 ± 2,851 IU/L, *P* = 0.045) and a higher peak level of HBV DNA (6.7 ± 8.5 versus 3.4 ± 6.5 log copies/mL, *P* < 0.0001). A significantly higher percentage of genotype A patients were treated with NAs (57% versus 40%, *P* = 0.013). These data are summarized in Table 1.

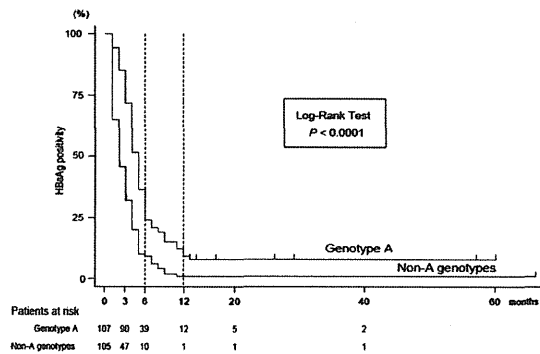


Fig. 1. Comparison of the cumulative proportion of AHB patients maintaining HBsAg positivity between genotype A and non-A genotypes, analyzed using the Kaplan-Meier test. $P < 0.0001$, genotype A: red line, non-A genotypes: blue line.

Cumulative Maintenance of HBsAg Positivity During Follow-up in Patients With Genotype A and Non-A Genotypes.

In the patients infected with genotype A and non-A genotypes, the mean durations of HBsAg positivity maintenance were 6.7 ± 8.5 and 3.4 ± 6.5 months, respectively ($P < 0.0001$; Table 1, Fig. 1). For 6 months after AHB onset, the number of patients with genotype A and non-A genotypes maintaining HBsAg positivity were 39/107 (36.4%) and 10/105 (9.5%), respectively ($P < 0.001$). However, in many patients HBsAg disappeared between 7 and 12 months after AHB onset; that is, HBsAg disappeared in 31/107 (29.0%) of patients with genotype A and in 9/105 (8.6%) of patients with non-A genotypes during this time period. However, in some patients HBsAg never disappeared after persisting for more than 12

months following AHB onset. When chronicity after AHB was defined as the persistence of HBsAg for more than 12 months, chronicity developed in 7.5% (8/107) of patients with genotype A and in 0.9% (1/105) of patients with non-A genotypes ($P = 0.018$).

Comparison of Characteristics Between Patients in Whom HBsAg Persisted More Than 6 or 12 Months and Those With Self-Limited AHB Infection.

Table 2 compares the demographic and clinical characteristics between patients in whom HBsAg disappeared within 6 months and those in whom HBsAg persisted for more than 6 months from AHB. The peak ALT levels ($1,882 \pm 2,331$ versus $1,018 \pm 696$ IU/L, $P = 0.0024$) and peak HBV DNA levels (6.3 ± 1.6 versus 7.4 ± 1.6 mg/dL, $P = 0.0004$) were significantly higher and lower in the former group than in the latter group, respectively. Moreover, marked differences were present in the distribution of genotypes between the two groups. The percentage of the HBV genotype A (46.1% versus 73.5%, $P = 0.003$) was significantly higher among patients in whom HBsAg was persistent for more than 6 months. In addition, we compared the demographic and clinical characteristics between patients in whom HBsAg disappeared within 12 months and those in whom HBsAg persisted for more than 12 months from AHB. Peak ALT ($1,787 \pm 2,118$ versus 775 ± 513 IU/L, $P = 0.0089$) and peak total bilirubin (8.7 ± 8.2 versus 3.8 ± 6.6 mg/dL, $P = 0.0039$) levels were significantly higher in the former group than in the latter group. In contrast, the peak HBV DNA levels (6.4 ± 1.6 versus 7.9 ± 1.4 mg/dL, $P = 0.0046$) were significantly lower

Table 2. Comparison Between Patients With Chronicity Following Acute Hepatitis B and Those With Self-Limited Acute Infections Determined by the Persistence of HBsAg for More Than 6 or 12 Months

Features	Persistence of HBsAg		P Value	persistence of HBsAg for More Than 12 Months		P Value
	Disappearance of HBsAg Within 6 Months (n = 178)	for More Than 6 Months From AHB (n = 34)		Disappearance of HBsAg Within 12 Months (n = 203)	From AHB (n = 9)	
Age (years)	38.2 ± 13.1	40.0 ± 14.5	0.454	38.1 ± 13.2	46.7 ± 14.0	0.061
Male sex	147 (82.6)	30 (88.2)	0.416	169 (83.3)	8 (88.9)	0.677
HBeAg positive	150 (84.3)	32 (94.1)	0.131	175 (86.2)	8 (88.9)	0.815
ALT (IU/L)	1882 ± 2331	1018 ± 696	0.0024	1787 ± 2118	775 ± 513	0.0089
Total bilirubin (mg/dL)	8.6 ± 7.5	8.7 ± 11.3	0.137	8.7 ± 8.2	3.8 ± 6.6	0.0039
HBV DNA (log copies/mL)	6.3 ± 1.6	7.4 ± 1.6	0.0004	6.4 ± 1.6	7.9 ± 1.4	0.0046
HBV genotype						
Non-A	96 (53.9)	9 (26.5)		104 (51.2)	1 (11.1)	
A	82 (46.1)	25 (73.5)	0.003	99 (48.8)	8 (88.9)	0.018
Sexual transmission	128/137 (93.4)*	24/26 (92.3)†	0.711	146/157 (93.0)‡	6/6 (100.0)§	0.356
NAs treatment (+)	82 (46.1)	21 (61.8)	0.093	98 (48.3)	8 (88.9)	0.017

Data are presented as n (%) and mean \pm SD. HBsAg, hepatitis B surface antigen; AHB, acute hepatitis B, HBeAg, hepatitis B e-antigen; ALT, alanine aminotransferase; HBV, hepatitis B virus; NAs, nucleotide analogs.

*Transmission routes of 41 patients were unknown.

†Transmission routes of 8 patients were unknown.

‡Transmission routes of 46 patients were unknown.

§Transmission routes of 3 patients were unknown.

Table 3. Multivariate Analysis of Factors Independently Associated With Persistence of HBsAg Positivity Following Acute Hepatitis B

Factors	Persistence of HBsAg More Than 6 Months From AHB		
	Odds Ratio	95% CI	P Value
ALT (per 1 IU/L increase)	1.000	0.999-1.000	0.035
HBV DNA (per 1 log copy/mL increase)	1.176	0.931-1.484	0.173
Genotypes			
Non-A	1.00		
A	4.224	1.853-9.631	0.001

95% CI, 95% confidence interval; ALT, alanine aminotransferase; HBV, hepatitis B virus.

in the former group than in the latter group. The percentages of HBV genotype A (48.8% versus 88.9%, $P = 0.018$) and NAs treatment (+) (48.3% versus 88.9%, $P = 0.017$) were significantly higher among patients in whom the HBsAg persisted for more than 12 months.

Factors Independently Associated With Viral Persistence Following AHB. A stepwise logistic regression model was used to perform multivariate analysis which explains relationships between some factors and persistence of HBsAg positivity more than 6 months following AHB. Peak ALT level, peak HBV DNA level, genotype A, and treatment with NAs were retained in the final multivariate logistic model in a backward stepwise manner ($P < 0.1$). For predicting the persistence of HBsAg for more than 6 months, only genotype A was independently associated with progression of AHB to the persistence of HBsAg (odds ratio [OR]: 4.224, $P = 0.001$, Table 3).

Characteristics of Patients Who Progressed to Chronicity That Was Defined as the Persistence of HBsAg for More Than 12 Months Following Acute Hepatitis B. Table 4 shows the clinical and virological characteristics of nine patients who progressed to

chronicity defined as the persistence of HBsAg for more than 12 months following AHB. Among the nine patients who progressed to chronicity from AHB, eight (88.9%) were men and eight (88.9%) were HBeAg-positive. In general, among the patients who progressed to chronicity following AHB, the peak HBV DNA levels were high, and the peak total bilirubin and ALT levels were low. In eight (88.9%) patients, entecavir was administered; however, the duration until the onset of NA treatment from AHB onset was long (75-570 days).

Early Onset of Treatment With NAs Was Able to Prevent Viral Persistence After AHB Caused by Genotype A. The cumulative proportion maintaining HBsAg positivity during follow-up, expressed in terms of time after AHB onset, were significantly longer in patients with NAs treatment than in those without NAs treatment ($P = 0.046$, Fig. 2A). Table 5 shows the percentages of patients in whom HBsAg persisted for more than 6 or 12 months among patients categorized based on the period of time (i.e., duration) until the onset of NAs treatment. For patients in whom the onset of NAs treatment was less than 4 weeks from the onset of AHB, 12.7% of the patients showed persistent HBsAg for more than 6 months, while none showed HBsAg positivity for more than 12 months. For patients in whom the onset of NAs treatment was at 5-8 weeks, 37.5% of the patients showed persistent HBsAg for more than 6 months, whereas none showed persistent HBsAg for more than 12 months. For all groups, the period of HBsAg positivity in patients starting NAs treatment within 8 weeks from AHB onset was significantly shorter than that in patients beginning NAs treatment after more than 8 weeks from AHB onset ($P < 0.0001$, Fig. 2B). Patients starting NAs treatment within 8 weeks from AHB onset never progressed to chronicity after AHB caused by genotype A.

Table 4. Characteristics of Patients Who Progressed to Chronicity Following Acute Hepatitis B

Case	Age	Gender	HIV	HBeAg	HBV DNA (log copies/mL)	Total Bilirubin (mg/dL)	ALT (IU/L)	Observation Period (Months)	NAs Treatment	Duration Until NAs Treatment (Days)	Transmission Routes	Genotype
1	23	Male	(-)	(+)	7.6	1.7	1271	26	ETV	570	Heterosexual	A
2	40	Male	(-)	(-)	8.8	1.4	568	13	ETV	240	Heterosexual	A
3	45	Male	(-)	(+)	7.7	0.9	867	57	ETV	135	Heterosexual	A
4	37	Male	(-)	(+)	7.6	3.4	384	29	ETV	75	Unknown	A
5	54	Male	(-)	(+)	9	2	455	17	ETV	155	Homosexual	A
6	45	Male	(-)	(+)	4.8	21.2	512	60	(-)	(-)	Homosexual	A
7	61	Male	(-)	(+)	9.1	1.5	804	17	ETV	88	Unknown	A
8	56	Male	(-)	(+)	9.0	1.1	1820	14	ETV	118	Unknown	A
9	31	Female	(-)	(+)	7.4	0.8	296	66	ETV	150	Blood transfusion	C

HIV, human immunodeficiency virus; HBeAg, hepatitis B e-antigen; HBV, hepatitis B virus; ALT, alanine aminotransferase; NAs, nucleotide analogs; ETV, entecavir.

Table 5. Proportion of Patients in Whom HBsAg Persisted for More Than 6 or 12 Months Among Patients Categorized Based on the Number of Weeks Until the Onset of NAs Treatment

Duration Until Onset of NAs Treatment (Weeks)	Persistence of HBsAg for More Than 6 Months	Persistence of HBsAg for More Than 12 Months	Total Patients
<4 weeks (n, %)	9 (12.7)	0 (0)	71
5-8 weeks (n, %)	6 (37.5)	0 (0)	16
9-12 weeks (n, %)	1 (33.3)	1 (33.3)	3
13-16 weeks (n, %)	4 (100)	1 (25.0)	4
>17 weeks (n, %)	9 (100)	6 (66.7)	9
Total	29	8	103

HBsAg, hepatitis B surface antigen; NAs, nucleotide analogs.

Discussion

A multicenter nationwide study was conducted throughout Japan to evaluate the influence of clinical and virological factors on chronic outcomes in Japanese patients who contracted AHB in adulthood. The study was feasible in Japan, where a universal vaccination program for HBV has not been implemented because of the extremely high efficacy of the immunoprophylaxis that is given to babies born to carrier mothers. The implementation of this program has resulted in a decrease in the persistent HBV carrier rate from 1.4% to 0.3%.¹⁹ Selective vaccination means that Japanese are more likely to be infected with HBV by way of horizontal transmission since the percentage of the population possessing anti-HBs is much lower than that in countries in which universal vaccination programs have been established.²⁰ In addition, Japan is faced with the ever-increasing impacts of globalization: as many as 17 million Japanese travel abroad and over 7 million people

visit Japan from overseas each year. This “population mixing” may help to explain the increased prevalence in Japan of AHB due to genotype A, which is transmitted through indiscriminate sexual contact. Consequently, Japan may be the only country in the world where the influences of HBV genotypes, including genotype A (as is predominant in Western countries) and genotypes B and C (as are predominant in Asian countries), on chronic outcomes after AHB can be compared.

Currently, the persistence of HBsAg in serum for more than 6 months is considered to represent a progression to chronic infection.²¹ However, our data showed that HBsAg frequently disappeared between 7 to 12 months after the onset of AHB in patients with genotype A (31/107 [29.0%]) and non-A genotypes (9/105 [8.6%]) (Fig. 1). These patients were considered to exhibit prolonged cases of AHB, rather than persistent infection. This finding reflects the higher sensitivity of the most up-to-date assays for HBsAg as compared with previous methods. In the present study, HBsAg was measured by CLIA, which has been reported to be about 150 times more sensitive in the detection of HBsAg than reverse passive hemagglutination (RPHA)-HBsAg, which has been used for the last 30 years in Japan.²² The use of a more sensitive assay for HBsAg results in a longer period during which HBsAg may be detected. In this study, HBsAg did not disappear in nine patients after remaining continuously detectable for more than 12 months. Therefore, the persistence of HBsAg for more than 12 months, as measured with a highly sensitive method for detecting HBsAg, may be suitable for defining the progression of AHB to chronicity; however, further study is necessary to determine whether this definition is appropriate worldwide.

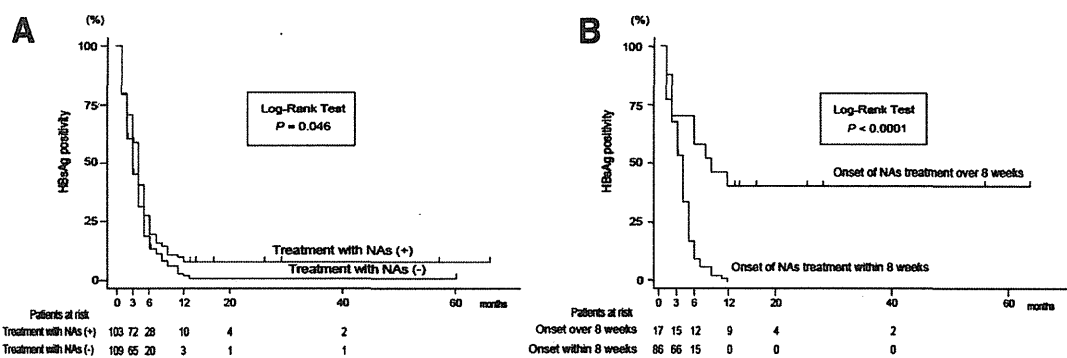


Fig. 2. (A) Comparison of the cumulative proportion of AHB patients maintaining HBsAg positivity between treatment with NAs (+) and treatment with NAs (-), as analyzed using the Kaplan-Meier test. $P = 0.046$, treatment with NAs (+): red line, treatment with NAs (-): blue line. (B) Comparison of the cumulative proportion of AHB patients in genotype A maintaining HBsAg positivity between treatment onset with NAs within 8 weeks and treatment onset with NAs over 8 weeks after onset of AHB, as analyzed using the Kaplan-Meier test. $P < 0.0001$, treatment onset with NAs over 8 weeks: red line, treatment onset with NAs within 8 weeks: blue line.