discontinuation. Because productions of HBsAg and HBcrAg are regulated by different promoter and enhance systems of HBV genome, their clinical values vary.

Follow-up method after discontinuation and conditions for retreatment

Follow-up after discontinuation of NUCs includes periodical measurement of HBV DNA levels (real-time PCR) and ALT levels. This study revealed that relapse after discontinuation occurs mostly within a year, gradually decreases after a year and rarely occurs after the first three years of discontinuation.[6] Therefore, we determined it necessary to pay attention especially to relapse immediately after discontinuation. In particular, we determined that it is desirable to follow up patients by blood tests at every two weeks up to 16 weeks after discontinuation and every four weeks after 16 weeks.

One of the important points is what the definition of hepatitis relapse is and how to follow up after discontinuation. Transient abnormalities in the ALT level or the HBV DNA level may be observed in about two-thirds patients who would finally achieve the inactive carrier state. Therefore, even if the ALT level or the HBV DNA level shows mild elevations, it is possible to follow up without retreatment. However, no criteria have been identified about when to discontinue follow-up and start retreatment. We assessed the transitions of ALT levels and HBV DNA levels after discontinuation of NUCs by the mean and maximum values to identify the criteria. From this assessment, a strong correlation was shown between the mean and the maximum value in both of them (Fig. 5).[6] Results of the ROC analysis revealed that the mean ALT of 30 IU/L corresponded to the maximum ALT of 79 IU/L and the mean HBV DNA of 4.0 log copies/m*l* corresponded to the maximum HBV DNA of 5.7 log

copies/m*l*. Patients with the ALT value of not less than 80 IU/L after discontinuation are highly likely to show the mean value of more than 30 IU/L and not assumed to finally meet the criteria for successful discontinuation. Similarly, Patients with the HBV DNA value of not less than 5.8 log copies/ml after discontinuation are most likely to show the mean value of more than 4.0 log copies/ml and not assumed to meet the criteria for successful discontinuation. Based on these results, we established the condition that patients with the ALT value of not less than 80 IU/L or the HBV DNA level of not less than 5.8 log copies/ml are less likely to finally achieve the inactive carrier state and should be considered retreatment with NUCs. It is considered that NUCs can be discontinued more efficiently and specifically in this condition. Physicians can use more severe criteria at their own discretion in consideration of safety. Less strict criteria can also be used, but it is recommended that the treatment should be done under a certain policy and do not follow the treatment without any aims.

Key points and future issues

This may be the first guideline for discontinuation of NUCs. Most of the data used in this guideline are retrospective and some points remain unsolved. Over 90% of the patients enrolled had genotype C and over 90% of cases were treated with lamivudine until discontinuation. [6] Therefore, key points and future issues are summarized in a section (Table 1-V). This guideline provides information to support physicians to decide NUCs discontinuation timing but physicians should actually consider for each patient whether NUCs can be discontinued or not because long-term prognosis after NUC discontinuation is not yet clear enough and patients' wishes and physicians' decision need to be prioritized. When NUCs cannot be successfully

discontinued, one of the options is re-administration of NUCs. However, it is not investigated whether re-administration of NUCs result the emergence and development of resistant strains. Further, it is not resolved which NUC should be given when re-administration is required. The consent from patients will be necessary on these points.

One of the issues to be investigated in the future is to improve accuracy in predicting hepatitis relapse after discontinuation. Investigations on the following approaches are suggested; higher sensitive HBV DNA , HBV RNA,[12, 13] HBV genotypes and HBV genetic mutations. Since these guidelines were prepared based on retrospective studies, it is necessary to validate them with prospective studies. In addition, how to actively discontinue NUCs by sequential treatment with interferon should also be included as an important issue to be investigated.

Three kinds of NUCs are available now in Japan. Lamivudine is the first NUC introduced into Japan in 2000. Adefovir dipivoxil is used mainly for patients with lamivudine resistance. Entecavir is now recommended as the first choice NUC. Over 10 years have passed since the first NUC became available in Japan and this is the first full-scale guideline for NUC discontinuation. Although this guideline may not be completely sufficient and needs further investigations, this is the first step to start, leading to a better one in the future.

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Table 1 Guidelines for avoiding risks resulting from discontinuation of nucleos(t)ide analogues 2012

I. Aims of these guidelines

In treatment with NUCs in patients with chronic hepatitis B, it is one of important treatment goals to aim at drug-free by discontinuation of NUCs. However, discontinuation of NUCs often results in hepatitis relapse which may become severe. Sufficient consideration must be given to the risk in case of discontinuation.

HBs antigen negative is the goal of treatment with NUCs, but it can't be always achieved easily. Therefore, discontinuation may be considered even if HBs antigen remains positive. These guidelines aim to discontinue NUCs in such conditions and finally achieve the inactive carrier state (ALT<30 IU/L and HBV DNA level in blood<4.0 log copies/m*l*).

It is currently unknown which of the two options about NUCs, discontinuation or continuation, is effective on life prognosis or liver carcinogenesis. We established these guidelines to be referred in case of considering discontinuation due to various reasons. We aimed to identify patients with a high possibility of successful discontinuation or patients who should inversely continue the treatment and establish indicators for follow-up after discontinuation to avoid risks resulting from discontinuation of NUCs as much as possible.

II. Requirements to avoid risk of developing severe hepatitis resulting from relapse

The following requirements are determined for discontinuation to previously assume and avoid the risk of developing severe hepatitis.

1. Both of the doctor and the patient fully understand the risk of a high frequency of hepatitis relapse that may become severe.
2. It is possible to follow up as well as to treat appropriately in case of relapse. (Involvement of a specialist is recommended.)
3. The patient has mild hepatic fibrosis with good hepatic functional reserve and will not easily

develop severe hepatitis in relapse. (NUCs should not be discontinued in patients with hepatic cirrhosis or chronic hepatitis with progressed fibrosis similar to cirrhosis)

III. Assessment of proliferative potential of HBV and conditions to reduce the relapse risk

1. Requirements for discontinuation of nucleos(t)ide analogues

Almost all patients with high proliferative potential of HBV will relapse after discontinuation. It is essential not to discontinue NUCs in these patients and the requirements were determined as follows:

Requirements for discontinuation
◇ HBV DNA level in blood is negative (real-time PCR) at the time of discontinuation
◇ HBe antigen level in blood is negative at the time of discontinuation

2. Condition for duration of treatment period of NUCs

Since short-term treatment with NUCs can easily result in relapse, it is recommended to meet the following condition.

Condition for duration of treatment period
◇ More than 2 years after the initial administration of NUCs

3. Assessment of relapse risk by scoring of viral antigen levels

For the patients who meet the requirements for discontinuation (HBV DNA negative and HBe antigen negative at the time of discontinuation), the HBsAg level and the HBcr antigen level at the time of discontinuation can be scored to predict the relapse risk by the following three groups based on the total score. This risk prediction aims to determine whether NUCs should be discontinued or not by reference to it to reduce the relapse risk.

HBsAg levels at the time of discontinuation	**Scores**	**HBcrAg levels at the time of discontinuation**	**Scores**
Less than 1.9 log IU/m*l* (less than 80 IU/m*l*)	0	Less than 3.0 log U/m*l*	0
1.9 - 2.9 log IU/m*l* (80-800 IU/m*l*)	1	3.0 - 4.0 log U/m*l*	1
Not less than 2.9 log IU/m*l* (not less than 800 IU/m*l*)	2	Not less than 4.0 log U/m*l*	2

Relapse risk	**Total scores**	**Percentage of prediction success**	**Assessment**
Low risk group	0	80% to 90%	Discontinuation can be considered. It is essential to pay attention to relapse because some patients with low risk may develop hepatitis relapse.
Medium risk group	1 - 2	Approx. 50%	Discontinuation can be considered depending on the situation. Further consideration is needed about conditions and the way to discontinue in the future.
High-risk group	3 - 4	10% to 20%	Continuous treatment is recommended. However, patients under 35 years old show a relatively higher rate of successful discontinuation of 30-40%.

IV. Follow-up method after discontinuation and conditions for retreatment

1. HBV DNA levels (real-time PCR) and ALT levels must be periodically measured after discontinuation of NUCs to pay attention to HBV proliferation and hepatitis relapse resulting from proliferation.
2. Relapse after discontinuation is mostly observed within a year and then gradually decreases. It is rare to relapse after the first three years. Therefore, it is necessary to pay attention to relapse immediately after discontinuation. In particular, patients should be followed up by blood tests at every two weeks up to 16 weeks after discontinuation and every four weeks after 16 weeks.
3. Transient abnormalities in ALT levels or HBV DNA levels may be observed in about two-thirds patients who successfully discontinued NUCs and would finally achieve the inactive carrier state. Therefore, even if the ALT level or the HBV DNA level shows mild elevations, it is possible to keep following up without retreatment. However, patients who meet the following condition are less likely to finally achieve the inactive carrier state and should be considered retreatment with NUCs.

Condition to consider retreatment with NUCs
◇ ALT ≧ 80 IU/L or HBV DNA ≧ 5.8 log copies/m*l* after discontinuation

V. Key points and future issues

1. The status differs in each patient. Objectives and significance also differ by patient. Thus doctors must determine whether NUCs should be discontinued or not in consideration of those conditions. In case of considering discontinuation, it is recommended to consult with a specialist of hepatic diseases.
2. In case of retreatment with NUCs due to hepatitis relapse after discontinuation, it is unknown whether it results in higher emergence of strains resistant to NUCs or not compared with

patients without discontinuation.

3. Since HBV carriers rarely experience hepatitis relapse even in the inactive carrier state (HBV DNA<4.0 log copy/m*l* and ALT<30 IU/L), they must be followed up after successful discontinuation. Liver carcinogenesis also requires follow-up.
4. The followings are included in future issues; improvement of accuracy in the criteria for discontinuation of NUCs; investigation of the criteria used in these guidelines in a prospective study; and investigation of the way to actively discontinue NUCs using sequential treatment with interferon.

Figure legends

Fig. 1. Replication process of HBV which originates from HBV cccDNA molecules pooled in nucleus of hepatocyte.

Fig. 2. Comparison of non-relapse rates using Kaplan-Meier method between 41 patients with serum HBV DNA not lower than 3.0 log copies/ml or with HBeAg and 85 patients with serum HBV DNA lower than 3.0 log copies and without HBeAg at the time of NUC discontinuation.

Fig. 3. Receiver operating characteristic curve (ROC) analysis of HBsAg and HBcrAg levels to discriminate between patients with and without hepatitis relapse. The existence of two inflection points is suggested for both HBsAg and HBcrAg levels. Short diagonal lines indicate main inflection points and short broken diagonal lines indicate second inflection points. Vertical lines indicate actual values of antigens that correspond to the main inflection points and vertical broken lines indicate actual values of antigens that correspond to the second inflection points.

Fig. 4. Comparison of non-relapse rates using the Kaplan-Meier method among 3 groups classified by the sum of the scores of HBsAg and HBcrAg levels at the time of NUC discontinuation.

Fig. 5. Correlation between maximal and mean levels of ALT (left) and HBV DNA (right) after discontinuation of NUCs. Open circles indicate patients with detectable HBeAg and closed squares indicate patients without detectable HBeAg.

Fig. 1

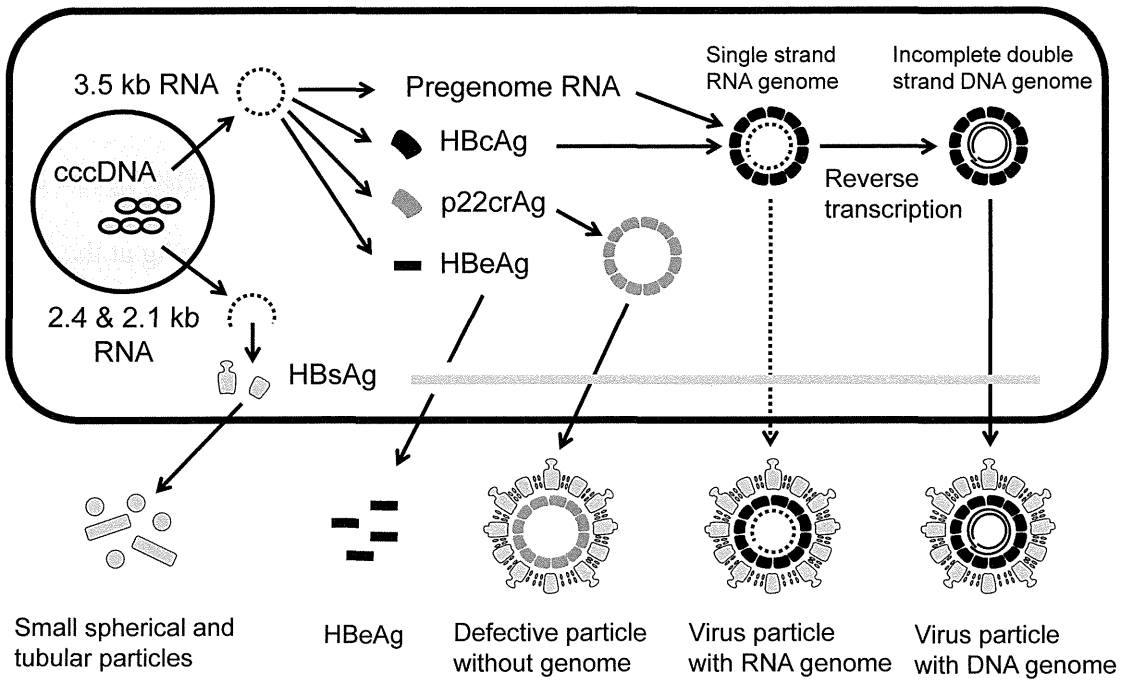

3.5 kb RNA
Pregenome RNA
Single strand RNA genome
Incomplete double strand DNA genome
cccDNA
HBcAg
p22crAg
HBeAg
Reverse transcription
2.4 & 2.1 kb RNA
HBsAg
Small spherical and tubular particles
HBeAg
Defective particle without genome
Virus particle with RNA genome
Virus particle with DNA genome

Fig. 2

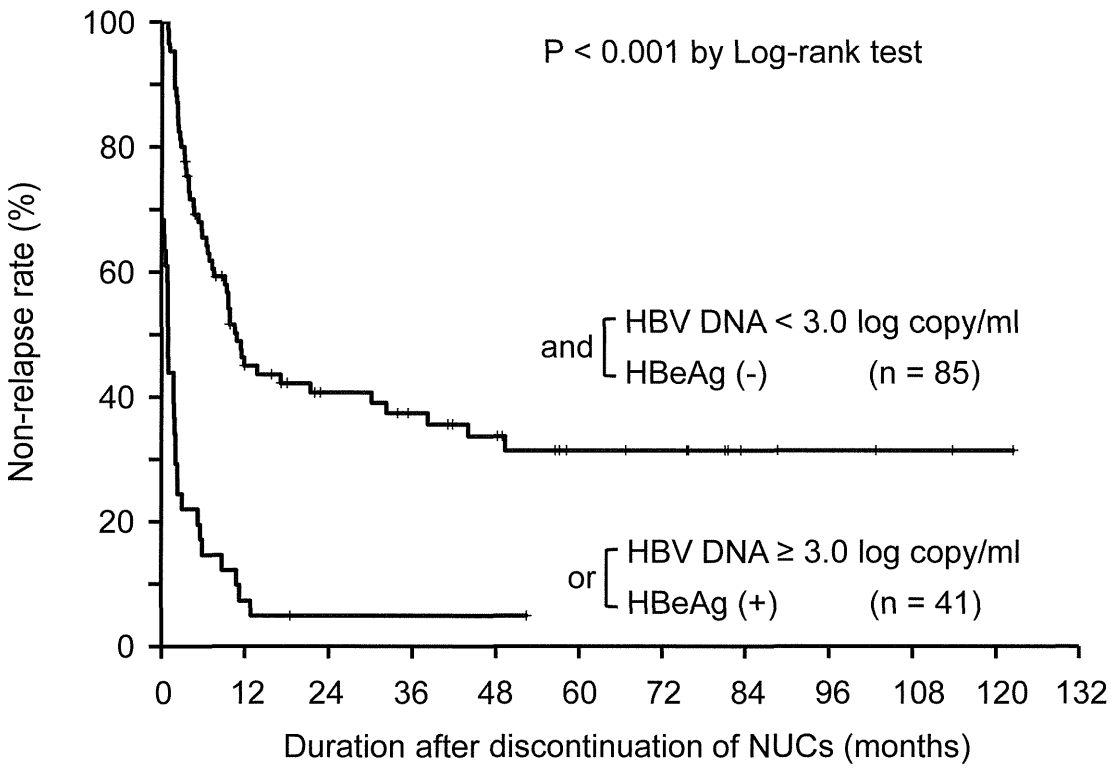

P < 0.001 by Log-rank test
Non-relapse rate (%)
100
80
60
40
20
0
and
HBV DNA < 3.0 log copy/ml
HBeAg (-)
(n = 85)
or
HBV DNA ≥ 3.0 log copy/ml
HBeAg (+)
(n = 41)
0
12
24
36
48
60
72
84
96
108
120
132
Duration after discontinuation of NUCs (months)

Fig. 3

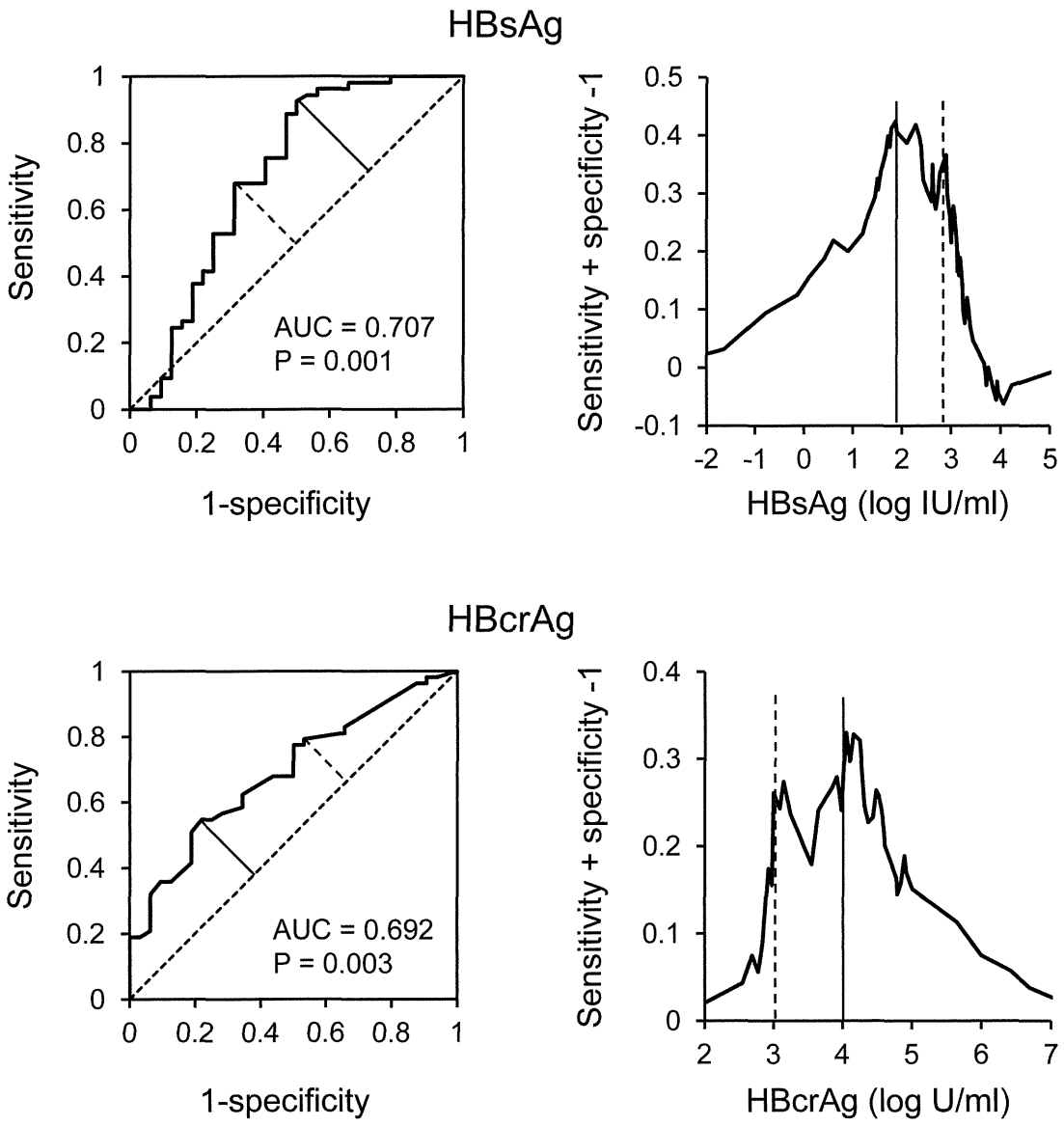

HBsAg
Sensitivity
1-specificity
AUC = 0.707
P = 0.001
Sensitivity + specificity -1
HBsAg (log IU/ml)
HBcrAg
Sensitivity
1-specificity
AUC = 0.692
P = 0.003
Sensitivity + specificity -1
HBcrAg (log U/ml)

Fig. 4

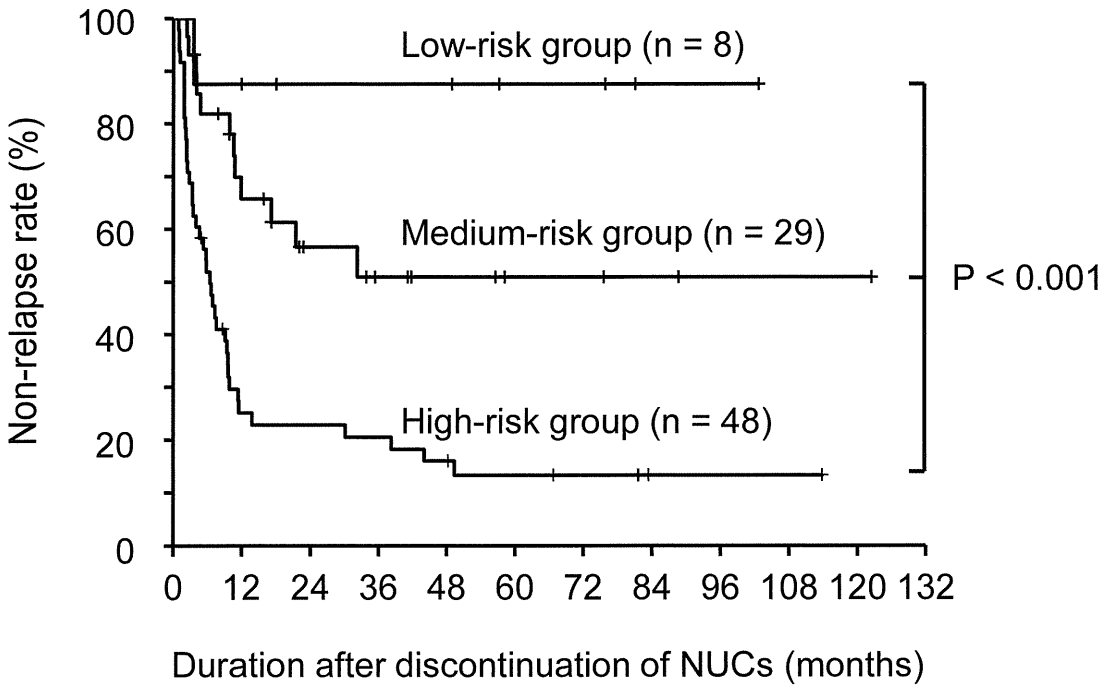
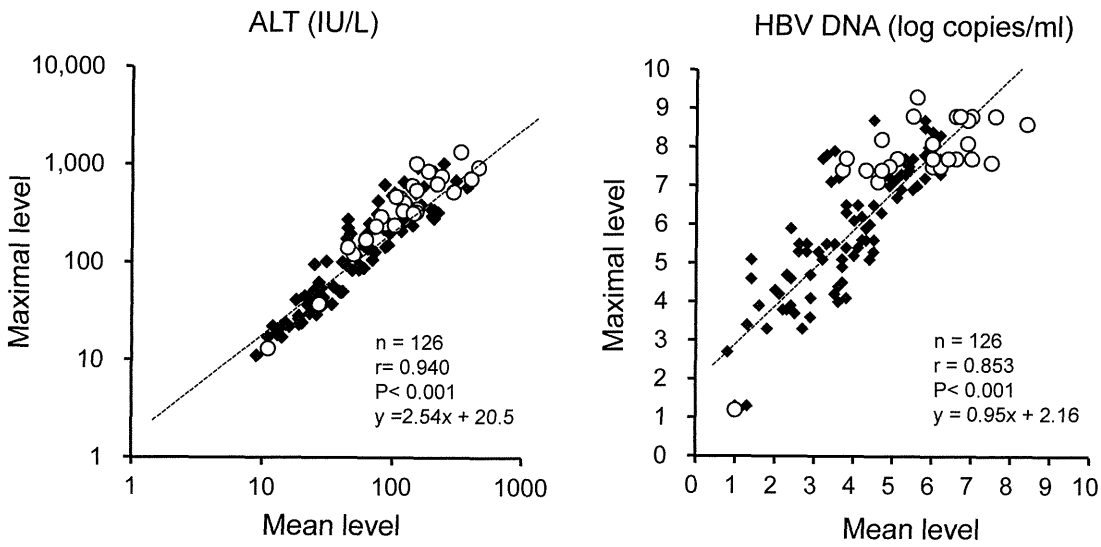

100
80
60
40
20
0
Non-relapse rate (%)
Low-risk group (n = 8)
Medium-risk group (n = 29)
High-risk group (n = 48)
P < 0.001
0 12 24 36 48 60 72 84 96 108 120 132
Duration after discontinuation of NUCs (months)

Fig. 5


ALT (IU/L)
Maximal level
Mean level
10,000
1,000
100
10
1
1
10
100
1000
n = 126
r= 0.940
P< 0.001
y =2.54x + 20.5
HBV DNA (log copies/ml)
Maximal level
Mean level
0 1 2 3 4 5 6 7 8 9 10
n = 126
r = 0.853
P< 0.001
y = 0.95x + 2.16

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Changes in the serum level of hepatitis B virus (HBV) surface antigen over the natural course of HBV infection

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Abstract
Background Despite its status as a potential biomarker of hepatitis B virus (HBV) response to interferon treatment, the changes in hepatitis B surface antigen (HBsAg) levels over the natural course of HBV carriers have not been analyzed sufficiently.
Methods A total of 101 HBV carriers were followed prospectively from 1999 to 2009. HBsAg level was measured yearly during the followed period.
Results HBsAg levels at baseline ranged from −1.4 to 5.32 log IU/ml, with a median value of 3.2 log IU/ml. Lower HBsAg levels were significantly associated with higher age and lower HBV replication status. The rate of change of HBsAg levels showed two peaks, with a cut-off value of −0.4 log IU/year. Based on this, patients were tentatively classified into rapid decrease (rate of change <−0.4 log IU/year) and non-rapid decrease groups. All baseline levels of HBsAg, HB core-related Ag, and HBV DNA were lower in the rapid decrease group than in the non-rapid decrease group. Patients with persistently positive HBeAg were all classified into the non-rapid decrease group. In patients with persistently negative HBeAg, HBV DNA levels were significantly ($P = 0.028$) lower in the rapid decrease group than in the non-rapid decrease group.
Conclusions Lower baseline HBsAg levels were significantly associated with older age and lower viral activity. Both a loss of HBeAg detection as well as inactive replication of HBV are suggested to be fundamental factors contributing to a rapid decrease in HBsAg over the natural course of HBV infection.

Keywords Hepatitis B virus · Hepatitis B surface antigen · Hepatitis B core-related antigen · Serum level · Natural course

Abbreviations

HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
IU	International unit
HBcrAg	Hepatitis B virus core-related antigen
HCC	Hepatocellular carcinoma
NA	Nucleos(t)ide analogue
HBeAg	Hepatitis B e antigen
CLEIA	Chemiluminescent enzyme immunoassay
Da	Dalton
HR	Hazard ratio
cccDNA	Covalently closed circular DNA

Introduction

With an estimated 350–400 million cases of chronic infection, hepatitis B virus (HBV) infection is a major worldwide health problem [1]. Chronic infection of HBV often leads to chronic hepatitis and eventually to liver cirrhosis and hepatocellular carcinoma [2, 3]. During infection, hepatitis B surface antigen (HBsAg), which is a component of the virion envelope, is secreted into the

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bloodstream in large amounts as subviral particles. Thus, serum HBsAg is routinely used as a marker for detection of HBV infection.

Recently, several groups have reported that HBsAg levels can be used as an indicator of the response to peg-interferon in chronic hepatitis B similarly to the conventional markers of HBV DNA level and hepatitis B (HB) e antigen/antibody status [4, 5]. Since HBV carriers who clear HBsAg usually have a better prognosis than those who do not [6–8], it may be worthwhile to monitor HBsAg levels in the natural disease course of HBV infection. However, such changes need to be clarified more thoroughly to validate their clinical significance. In the present study, we analyzed the changes in HBsAg levels in a cohort of HBV carriers who were followed prospectively and compared them with those of HBV DNA and HB core-related antigens (HBcrAg) levels.

Patients and methods

Patients

A total of 101 HBV carriers were followed prospectively from 1999 to 2009. Patients were selected consecutively between 1997 and 1999 and met the following conditions: (1) HBsAg was positive in at least two examinations performed over 1 year apart; (2) no complications of hepatocellular carcinoma (HCC) or signs of hepatic dysfunction, such as jaundice or ascites, were observed; (3) nucleos(t)ide analogues (NAs) were not administered at the start of follow-up; and (4) patients were negative for hepatitis C and human immunodeficiency virus antibodies. The clinical and virological characteristics of our cohort are shown in Table 1. The 101 patients consisted of 57 men and 44 women with a median age of 50 years (range 15–83 years). Hepatitis B e antigen (HBeAg) was positive in 38 (38%) patients and negative in 63 (63%). Of the 38 patients with HBeAg, 15 remained positive and 23 became negative during the follow-up period. Alanine aminotransferase (ALT) level flares of over 1,000 IU/L were observed in four (17%) of the 23 patients with HBeAg loss, but in none of the 15 patients with persistent HBeAg ($P = 0.138$). HBV genotype distribution was A in three (3%) patients, B in nine (9%), C in 87 (86%), and undetermined in two (2%). All patients were seen at Shinshu University Hospital or one of its affiliated hospitals. Our cohort tended to have a higher prevalence of cirrhosis (19%) and HCC (14%). These tendencies may be attributed to the higher age distribution in our cohort than that in other cohorts of HBsAg studies [6, 9, 10].

Patients were seen at least once a year during the 10 years of follow-up. The presence of cirrhosis was judged by histological findings and/or typical findings seen in cirrhosis, such as esophageal varices and splenomegaly. Screening for HCC was done using ultrasonography (US), computed tomography (CT), and/or magnetic resonance (MR) imagining at least once a year. The presence of complicating HCC was judged by evidence of characteristic hepatic masses on liver CT, MRI, and/or hepatic angiography. Serum samples were collected on a yearly basis and immediately stored at −20°C or below until assayed. This study was approved by the Ethics Committee of Shinshu University.

Hepatitis B viral markers

Serological markers for HBV, including HBsAg, HBeAg, and HBe antibody, were tested using commercially

Table 1 Clinical and virological characteristics of patients with respect to HBeAg status

Characteristic	Overall ($n = 101$)	HBeAg-positive ($n = 38$)	HBeAg-negative ($n = 63$)	P
At baseline				
Age (years)[a]	50 (15 to 83)	42 (15 to 72)	53 (25 to 83)	<0.001
Male[b]	57 (56%)	22 (58%)	35 (56%)	>0.2
With cirrhosis[b]	19 (19%)	10 (26%)	9 (14%)	0.188
ALT (IU/L)[a]	31 (10 to 447)	47 (13 to 447)	29 (10 to 81)	0.002
HBV genotype (A:B:C:UD)	3:9:87:2	1:0:36:1	2:9:51:1	0.144
HBsAg (log IU/ml)[a]	3.2 (−1.4 to 5.3)	3.7 (1.6 to 5.3)	2.9 (−1.4 to 4.3)	<0.001
HBcrAg (log U/ml)[a]	3.8 (<3.0 to >6.8)	6.8 (<3.0 to >6.8)	3.1 (<3.0 to >6.8)	<0.001
HBV DNA (log copies/ml)[a]	4.7 (neg. to >9.5)	7.4 (2.4 to >9.5)	3.6 (neg. to 8.3)	<0.001
During follow-up				
Followed period (years)[a]	5 (1 to 10)	6 (1 to 10)	5 (1 to 10)	>0.2
Clearance of HBsAg[b]	20 (20%)	3 (8%)	17 (27%)	0.022
Complication of HCC[b]	14 (14%)	8 (21%)	6 (10%)	0.139
Introduction of NAs[b]	23 (23%)	11 (29%)	12 (19%)	>0.2

UD undetermined

[a] Data are expressed as median (range)

[b] Data are expressed as positive number (%)