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Guidelines for avoiding risks resulting from discontinuation of nucleos(t)ide analogues in patients with chronic hepatitis B

Short running title: Guidelines to discontinue NUCs

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Key words: nucleos(t)ide analogue, discontinuation of treatment, hepatitis B, hepatitis relapse, HBV cccDNA

Abstract

Since nucleos(t)ide analogues (NUC) can lead to rapid reduction in HBV DNA levels in blood and normalization of alanine aminotransferase levels in many patients. They also provide histological improvement which results in a reduction in liver carcinogenesis. However, it is difficult to completely remove viruses even by NUCs and there are some problems such as emergence of resistant strains and hepatitis relapse resulting from discontinuation of treatment. One of the reasons is that NUCs reduce the HBV DNA level in blood but have almost no effects on the HBV cccDNA level in hepatocyte nuclei which are the origins of HBV replication and HBV cccDNA remains for a long period.

For treatment with NUCs in patients with hepatitis B, it is considered that NUCs should not be easily discontinued since discontinuation often results in hepatitis relapse. However, it has not been clearly revealed when and how hepatitis relapses after discontinuation. Although there are not a few patients without hepatitis relapse after discontinuation or with mild relapse and finally in a stable condition, it has not been established how to identify such patients efficiently.

We performed research to investigate characteristics of the course after discontinuation of treatment and definition of hepatitis relapse and estimate the relapse rate. “Guidelines for avoiding risks resulting from discontinuation of NUCs 2012,” is summarized based on the study results. Because the guidelines are written in Japanese, we explain those in English as a review article.

Introduction

Since nucleos(t)ide analogues (NUC) recently introduced to treatment of hepatitis B strongly inhibit proliferation of HBV, they can lead to rapid reduction in HBV DNA levels in blood and normalization of alanine aminotransferase (ALT) levels in many patients.[1] They also provide histological improvement which results in a reduction in liver carcinogenesis[2, 3] and can be orally administered with few side effects, so they are widely used in clinical practice. However, it is difficult to completely remove viruses even by NUCs and there are some problems such as emergence of resistant strains and hepatitis relapse resulting from discontinuation of treatment.[4] One of the reasons is that NUCs reduce the HBV DNA level in blood but have almost no effects on the HBV cccDNA level in hepatocyte nuclei which are the origins of HBV replication and HBV cccDNA remains for a long period.[5]

For treatment with NUCs in patients with hepatitis B, it is considered that NUCs should not be easily discontinued since discontinuation often results in hepatitis relapse. However, it has not been clearly revealed when and how hepatitis relapses after discontinuation. Although there are not a few patients without hepatitis relapse after discontinuation or with mild relapse and finally in a stable condition, it has not been established how to identify such patients efficiently.

We performed research funded by the Health and Labour Sciences Research Grant to investigate characteristics of the course after discontinuation of treatment and definition of hepatitis relapse and estimate the relapse rate.[6] “Guidelines for avoiding risks resulting from discontinuation of NUCs 2012,” is summarized based on the study results (Kanzo 2012; 53: 237-242, The Japan Society of Hepatology). The guidelines don’t always recommend discontinuation of NUCs. We determined them to be referred

if it is necessary to consider discontinuation due to various reasons.

Serum markers reflecting amount of HBV cccDNA in hepatocytes

The replication process of HBV in hepatocytes is shown in Fig. 1. HBV is an enveloped DNA virus containing a relaxed circular DNA genome converted into a covalently closed circular DNA (cccDNA) episome in the nucleus of infected cells. [7-10] These cccDNA molecules serve as transcriptional templates for production of viral RNAs that encode both viral structural and non-structural proteins. Hepatitis B surface antigen (HBsAg) is transcript from 2.1 kb and 2.4 kb mRNAs. On the other hand, hepatitis B core antigen (HBcAg), p22cr antigen (p22crAg)[11], and hepatitis B e antigen (HBeAg) are transcript from 3.5 kb mRNA which also serves as pregenome RNA. HBeAg is secreted into blood stream as a secretion protein, and p22crAg forms genome-negative core particles. HBcAg forms nucleocapsid particles by incorporating pregenome RNA. Once the pregenome RNA is reverse transcripted to DNA, the particles are enveloped with lipid layer containing HBsAg and then secreted into blood stream as virions.[8, 9] When the reverse transcriptation is inhibited by NUCs, virus particles with RNA genome are secreted instead of those with DNA genome.[12, 13]

HBV cccDNA is a stable molecule like chromosomal DNA which hardly be destroyed by DNases in natural conditions. Because NUCs are inhibitors of reverse transcriptase, they have no direct effect on reducing intrahepatic cccDNA levels. Therefore, reactivation of HBV replication which originates from HBV cccDNA and incidental hepatitis relapse occurs when NUCs are discontinued.

It is generally considered that HBV cccDNA levels in hepatocytes is well correlated with proliferative potential of HBV,[5] serum markers reflecting the cccDNA

level are suggested to be useful as clinical indicators. Serum level of HBV DNA correlates well with intrahepatic level of HBV cccDNA in natural course but not under NUC treatment. NUCs reduce serum level of HBV DNA rapidly by inhibiting the reverse transcription, but this inhibition does not reduce the cccDNA level.[5] On the other hand, serum levels of HBsAg and HB core-related antigen (HBcrAg) have been reported as markers reflecting cccDNA levels in hepatocytes even under NUC treatment.[14-17] HBcrAg assay measures all antigens coded by pre-core/core genome simultaneously which include HBcAg, HBeAg, and p22crAg, and has been reported to be useful for predicting clinical outcomes of patients who were treated with NUCs.[6, 17-22] HBsAg level is focused recently as a new marker and has been reported to be efficient in prediction of treatment effects by interferon and others.[14, 15]

Aims of these guidelines

These guidelines aim to identify patients with a higher possibility of successful discontinuation or patients who should continue treatments and avoid risks resulting from discontinuation of NUCs as much as possible by establishing indicators for follow-up after discontinuation (Table 1-I). Successful discontinuation in the guidelines is defined to finally achieve the inactive carrier state with the ALT level of less than 30 IU/L and the HBV DNA level in blood of less than 4.0 log copies/ml. These criteria were defined in compliance with the guidelines for treatment of chronic hepatitis B in Japan.[23] It is known that patients in the inactive carrier state show no progression of hepatic diseases and a reduction in the carcinogenic rate[24, 25] and the criteria are considered to be appropriate.

Requirements to avoid risk of developing severe hepatitis resulting from relapse

It is currently unable to predict hepatitis relapse after discontinuation of NUCs with a sufficient high accuracy. Therefore, we reviewed the risk of developing severe hepatitis and established requirements to prevent severe hepatitis (Table 1-II).[26] The presence of understanding about the risks of hepatitis relapse and severe hepatitis by both doctors and patients as well as the availability of a follow-up system after discontinuation and appropriate treatment for relapse are the basic essential requirements. Given patients with hepatic cirrhosis or chronic hepatitis with progressed fibrosis similar to cirrhosis can easily develop severe hepatitis and have higher risks of carcinogenesis in the future, we determined that those patients should not easily discontinue NUCs.

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

It has been experienced that patients with insufficient reduction of HBV DNA level or with HBeAg positive at the time of discontinuation of NUCs can develop hepatitis relapse in higher rate after discontinuation. The tendency was also confirmed scientifically in our study.[6] The cut-off value of HBV DNA level to predict hepatitis relapse was 3.0 log copies/mL by the ROC analysis. Almost all patients with higher HBV DNA levels or with HBeAg positive relapsed within a year while nearly 30% of patients with the HBV DNA levels less than 3.0 log copies/mL and without HBeAg were in the stable condition for a long period. (Fig. 2) Based on these results, we included sufficient reduction in HBV DNA levels and HBeAg negative in requirements for discontinuation. We determined the reference range of sufficient reduction in HBV

DNA levels in the actual guidelines not to be less than 3.0 log copies /ml but to be negative by real-time PCR in consideration of safety.

Factors relating to hepatitis relapse after discontinuation were analyzed in the population except for patients who were obviously predicted to relapse after discontinuation, or those with the HBV DNA levels of not less than 3.0 log copies/mL or HBeAg positive. The following factors were calculated to be significant; duration of treatment period of NUCs, HBsAg levels at the time of discontinuation, and HBcrAg levels at the time of discontinuation. Since the cut-off value in duration of treatment period was calculated as 16 months, we overestimated and established that NUCs should be discontinued more than 2 years after the initial administration in the guidelines.[6]

Two cut-off values were suggested from the results of the ROC analysis for the HBsAg and the HBcrAg levels at the time of discontinuation (Fig. 3); 1.9 and 2.9 log IU/m*l* for the HBsAg level and 3.0 and 4.0 log U/m*l* for the HBcrAg level. Based on this, HBsAg and HBcrAg levels were scored as shown in Table 1-III and three groups of the low risk group, the medium risk group and the high-risk group were determined. The percentage of prediction success was 80% to 90% in the low risk group, approximately 50% in the medium risk group and 10-20% in the high-risk group (Fig. 4). In further investigation of factors relating to hepatitis relapse in each group, no factors were newly found in the low and medium risk groups but age was a significant factor in the high-risk group. Although the percentage of prediction success rate is low in the high-risk group (10% to 20%), it resulted slightly higher which was from 30% to 40% with those patients younger than 35 years old.[6] It was interesting to find that the combination of HBsAg and HBcrAg levels were useful in preparing these guidelines for

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. 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.

Acknowledgments

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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.

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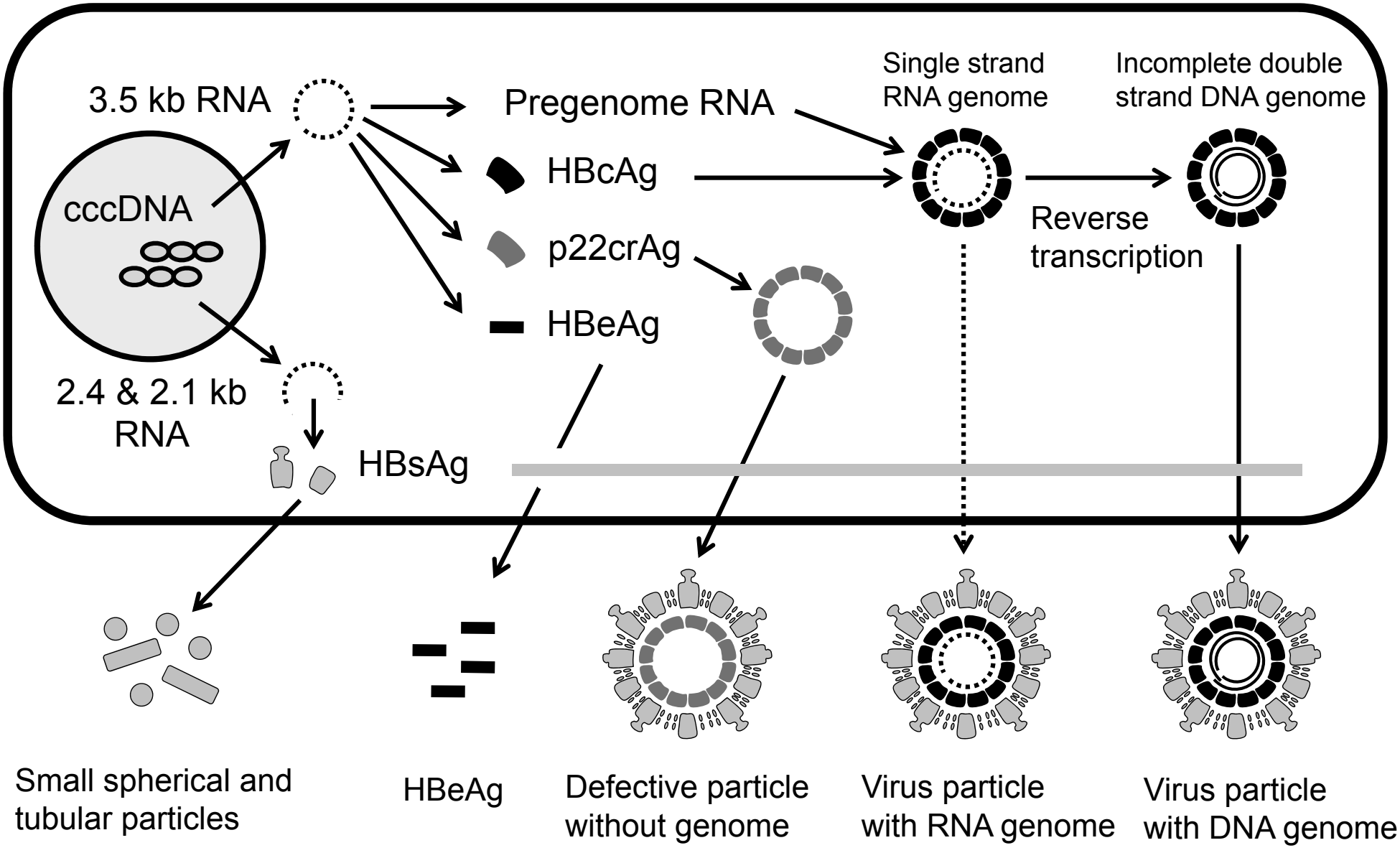
Fig.1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cccDNA
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HBcAg
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HBeAg
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Virus particle with RNA genome
Virus particle with DNA genome

Fig.2

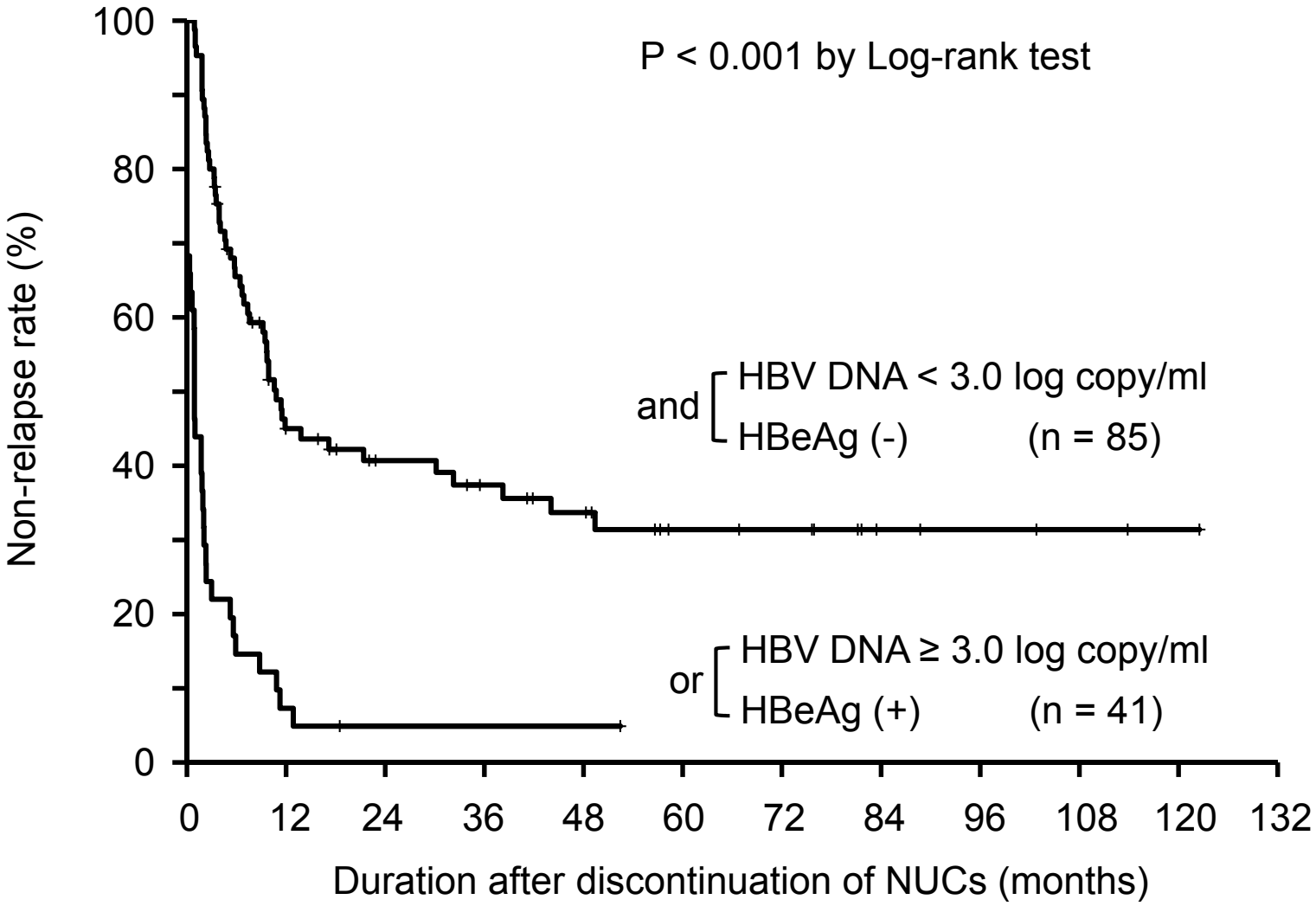

P < 0.001 by Log-rank test
and
HBV DNA < 3.0 log copy/ml
HBeAg (-)
(n = 85)
or
HBV DNA ≥ 3.0 log copy/ml
HBeAg (+)
(n = 41)
Non-relapse rate (%)
100
80
60
40
20
0
0
12
24
36
48
60
72
84
96
108
120
132
Duration after discontinuation of NUCs (months)

Fig.3

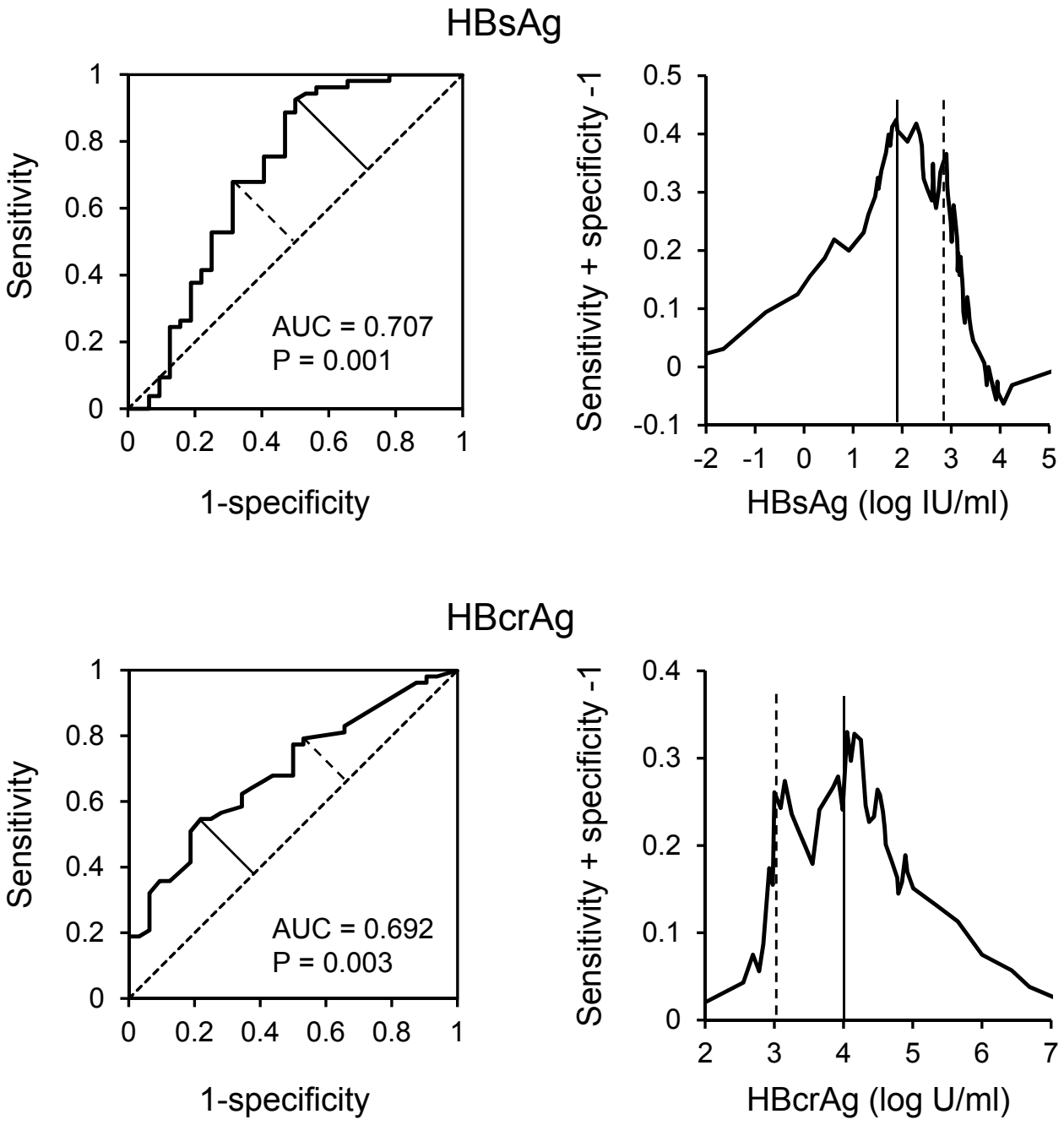

HBsAg
Sensitivity
1-specificity
AUC = 0.707
P = 0.001
Sensitivity + specificity -1
HBsAg (log IU/ml)
HBcrAg
Sensitivity
1-specificity
AUC = 0.692
P = 0.003
Sensitivity + specificity -1
HBcrAg (log U/ml)

Fig.4

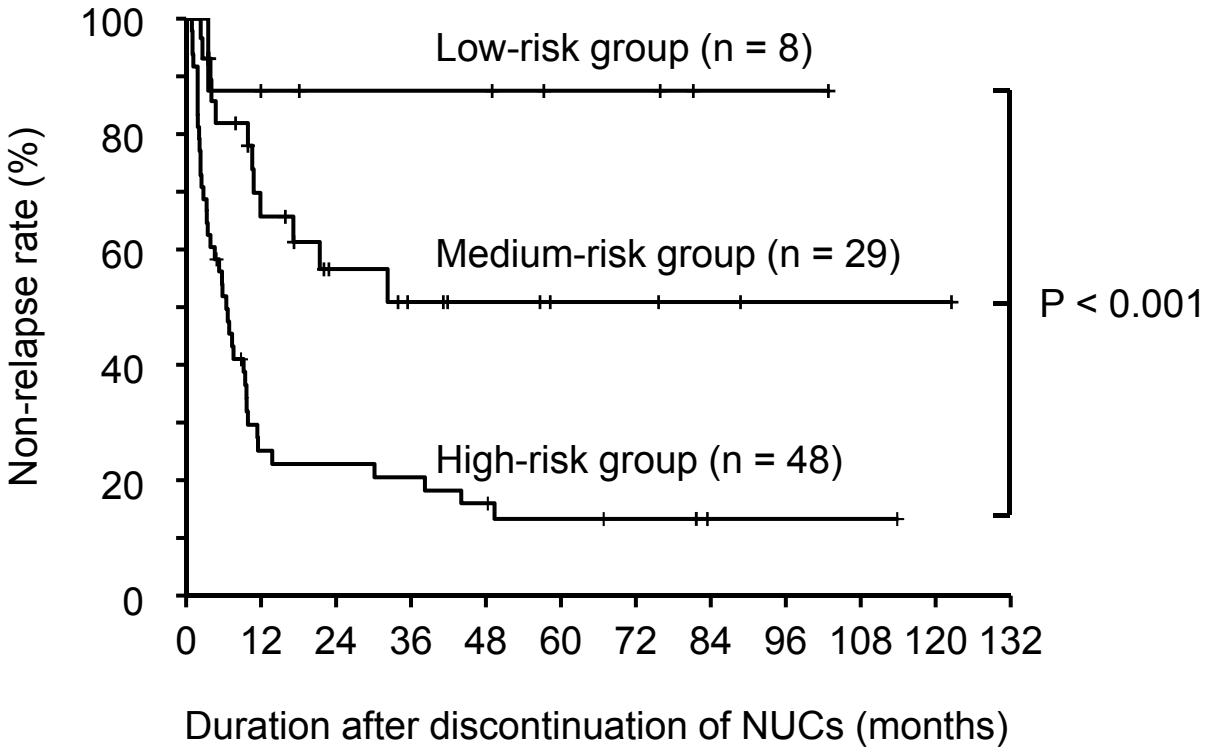
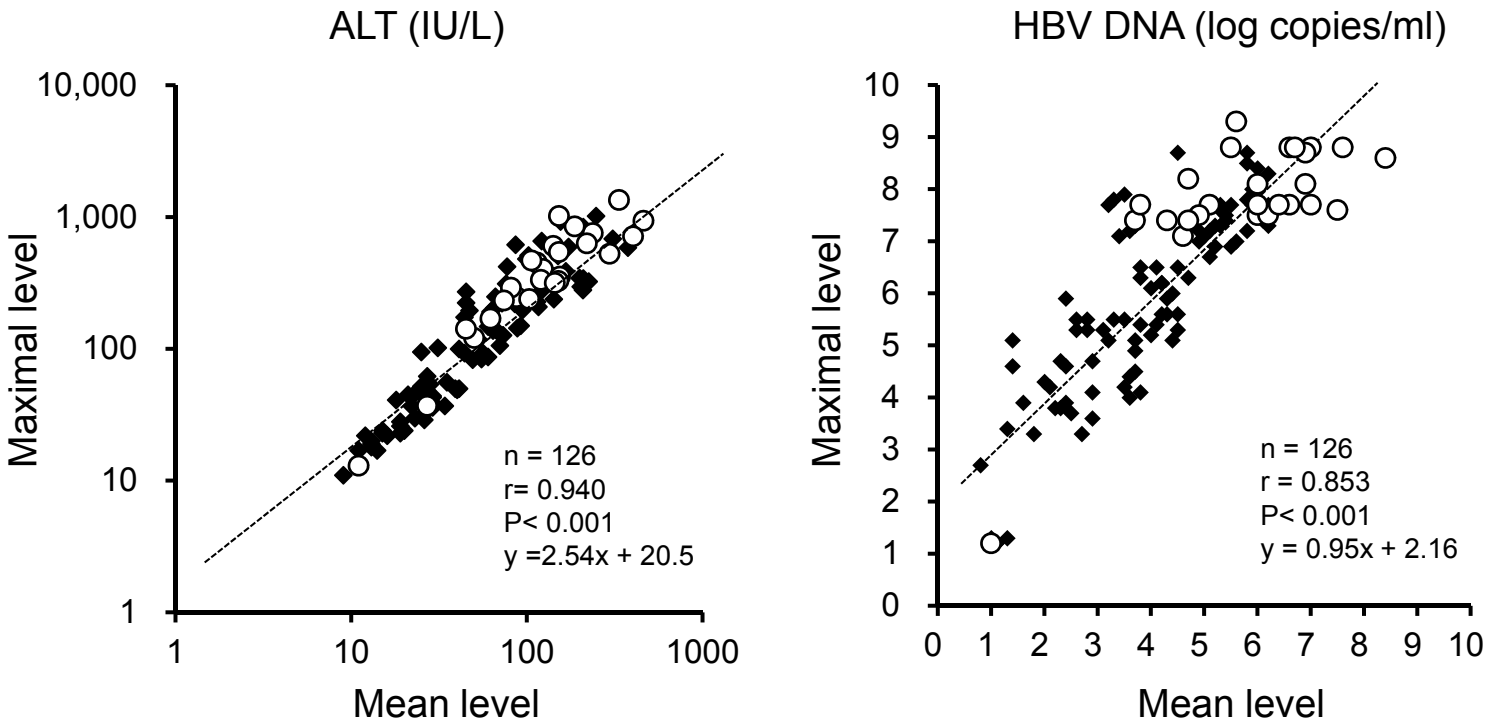

100
80
60
40
20
0
Non-relapse rate (%)
Low-risk group (n = 8)
Medium-risk group (n = 29)
High-risk group (n = 48)
P < 0.001
0 12 24 36 48 60 72 84 96 108 120 132
Duration after discontinuation of NUCs (months)

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