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Review Article

Guidelines for avoiding risks resulting from discontinuation of nucleoside/nucleotide analogs in patients with chronic hepatitis B

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Nucleoside/nucleotide analogs (NUC) can lead to rapid reduction in hepatitis B virus (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 NUC and there are some problems such as emergence of resistant strains and hepatitis relapse resulting from discontinuation of treatment. One of the reasons for this is that NUC 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 NUC in patients with hepatitis B, it is considered that NUC should not be easily discontinued because discontinuation often results in hepatitis relapse. However, it has not been clearly revealed when and how hepatitis relapses after discontinuation. Although some patients do not experience hepatitis relapse after discontinuation of NUC, or experience only mild relapse and finally achieve 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 them in English as a review article.

Key words: discontinuation of treatment, hepatitis B virus cccDNA, hepatitis B, hepatitis relapse, nucleoside/nucleotide analog

INTRODUCTION

BECAUSE NUCLEOSIDE/NUCLEOTIDE analogs (NUC) that have been recently introduced to treat hepatitis B strongly inhibit proliferation of hepatitis B virus (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 administrated p.o. with few side-effects, so they are widely used in clinical practice. However, it is difficult to completely remove viruses even by NUC and there are some problems such as emergence of resistant strains and hepatitis relapse resulting from discontinuation of treatment.[4] One of the reasons for this is that NUC 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 NUC in patients with hepatitis B, it is considered that NUC should not be easily discontinued because discontinuation often results in hepatitis relapse. However, it has not been clearly revealed when and how hepatitis relapses occur after discontinuation. Although some patients do not experience hepatitis relapse after discontinuation of NUC, or experience only mild relapse and finally achieve a stable condition, it has not been established how to identify such patients efficiently.

We performed research funded by a Health and Labor Sciences Research Grant to investigate characteristics of the course after discontinuation of treatment, definition of hepatitis relapse and estimation of relapse rate.[6] "Guidelines for avoiding risks resulting from discontinuation of NUCs 2012" is summarized based on the

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study results.[7] The guidelines do not always recommend discontinuation of NUC. We determined them to be referred to if it is necessary to consider discontinuation of NUC due to various reasons.

SERUM MARKERS REFLECTING AMOUNT OF HBV CCCDNA IN HEPATOCYTES

THE REPLICATION PROCESS of HBV in hepatocytes is shown in Figure 1. HBV is an enveloped DNA virus containing a relaxed circular DNA genome converted into a cccDNA episome in the nucleus of infected cells.[8–11] These cccDNA molecules serve as transcriptional templates for production of viral RNA that encode both viral structural and non-structural proteins. Hepatitis B surface antigen (HBsAg) is translated from 2.1-kb and 2.4-kb mRNA. On the other hand, hepatitis B core antigen (HBcAg), p22cr antigen (p22crAg)[12] and hepatitis B e-antigen (HBeAg) are translated from 3.5-kb mRNA which also serves as pregenome RNA. HBeAg is secreted into the 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 transcribed to DNA, the particles are enveloped with lipid layer containing HBsAg and then secreted into blood stream as virions.[9,10] When the reverse transcriptation is inhibited by NUC, virus particles with RNA genome are secreted instead of those with DNA genome.[13,14]

Hepatitis B virus cccDNA is a stable molecule like chromosomal DNA which can be barely destroyed by DNase in natural conditions. Because NUC 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 NUC are discontinued.

It is generally considered that HBV cccDNA levels in hepatocytes are well correlated with the 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 the natural course but not under NUC treatment. NUC 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 hepatitis B core-related antigen (HBcrAg) have been reported as markers reflecting cccDNA levels in hepatocytes even under NUC treatment.[15–18] HBcrAg assay measures all antigens coded by precore/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 NUC.[6,18–23] HBsAg level has received attention recently as a new marker and has been reported to be efficient in prediction of treatment effects by interferon and others.[15,16]

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

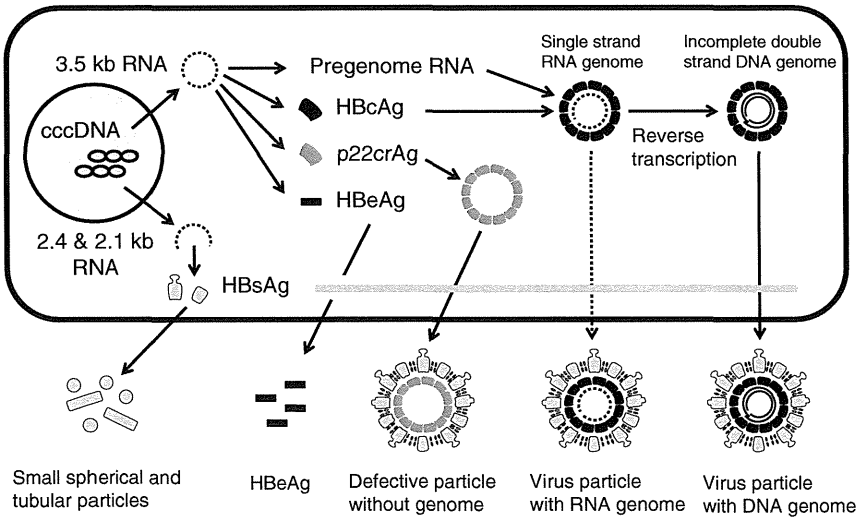

Figure 1 Replication process of hepatitis B virus (HBV) which originates from HBV cccDNA molecules pooled in nucleus of hepatocyte. HBcAg, hepatitis B core antigen; HBeAg, hepatitis B e-antigen; p22crAg, p22cr antigen.

risks resulting from discontinuation of NUC as much as possible by establishing indicators for follow up after discontinuation (Appendix 1-I). Successful discontinuation in the guidelines is defined as final achievement of the inactive carrier state with ALT level of less than 30 IU/L and 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.[24] It is known that patients in the inactive carrier state show no progression of hepatic diseases and a reduction in the carcinogenic rate[25,26] and the criteria are considered to be appropriate.

REQUIREMENTS TO AVOID RISK OF DEVELOPING SEVERE HEPATITIS RESULTING FROM RELAPSE

WE ARE CURRENTLY unable to predict hepatitis relapse after discontinuation of NUC with sufficient accuracy. Therefore, we reviewed the risk of developing severe hepatitis and established requirements to prevent severe hepatitis (Appendix 1-II).[27] The presence of understanding 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. Considering that 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 NUC.

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 NUC can develop hepatitis relapse at higher rates 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 receiver operating characteristic (ROC) analysis. Almost all patients with higher HBV DNA levels or were HBeAg positive relapsed within a year while nearly 30% of patients with HBV DNA levels less than 3.0 log copies/mL and without HBeAg were in a stable condition for a long period (Fig. 2). Based on these results, we included sufficient reduction in HBV DNA levels and

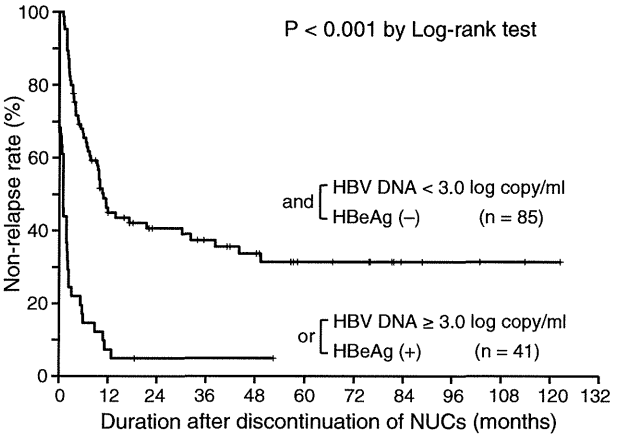

Figure 2 Comparison of non-relapse rates using Kaplan–Meier method between 41 patients with serum hepatitis B virus (HBV) DNA not lower than 3.0 log copies/mL or with hepatitis B e-antigen (HBeAg) and 85 patients with serum HBV DNA lower than 3.0 log copies and without HBeAg at the time of nucleoside/nucleotide analog (NUC) discontinuation.

HBeAg negativity 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 polymerase chain reaction (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 HBV DNA levels of not less than 3.0 log copies/mL or were HBeAg positive. The following factors were calculated to be significant: duration of treatment period of NUC; HBsAg levels at the time of discontinuation; and HBcrAg levels at the time of discontinuation. Because the cut-off value in duration of treatment period was calculated as 16 months, we overestimated and established that NUC 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 HBcrAg levels at the time of discontinuation (Fig. 3): 1.9 and 2.9 log IU/mL for the HBsAg level and 3.0 and 4.0 log U/mL for the HBcrAg level, respectively. Based on this, HBsAg and HBcrAg levels were scored as shown in Appendix 1-III and three groups – low-risk, medium-risk and high-risk – were determined. The percentage of prediction success was 80–90% in the low-risk group, approximately 50% in the medium-risk group and 10–20% in the high-risk

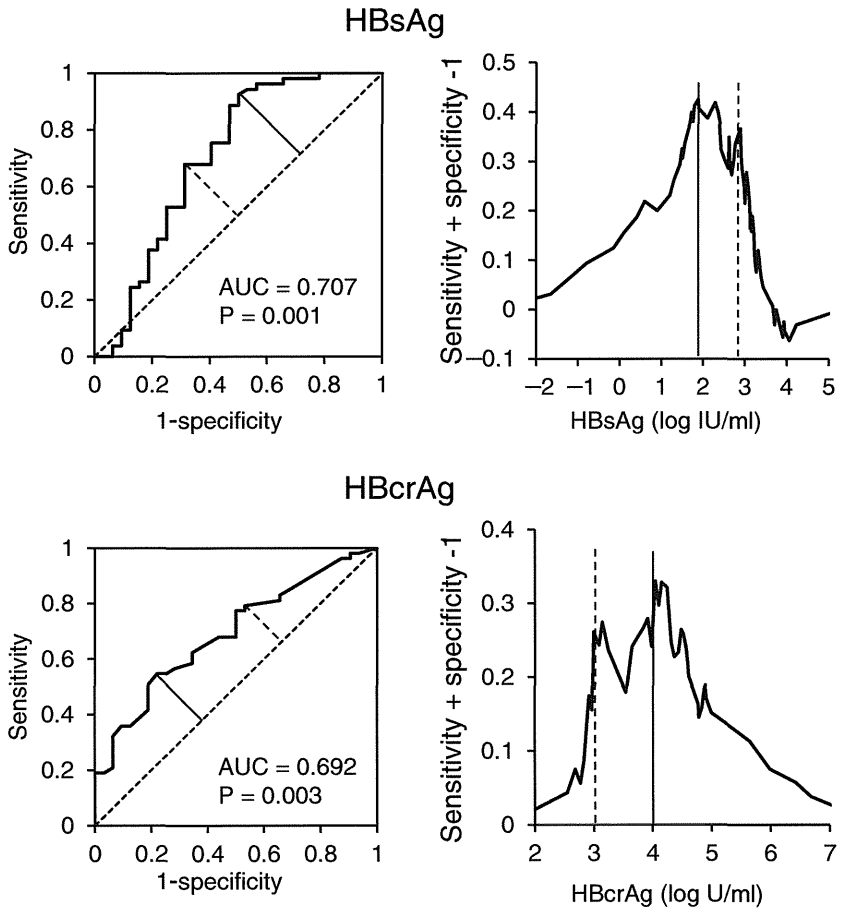

Figure 3 Receiver operating characteristic (ROC) analysis of hepatitis B surface antigen (HBsAg) and HB core-related antigen (HBcrAg) levels to discriminate between patients with and without hepatitis relapse. The existence of two inflection points is suggested for both HBsAg and HBcrAg levels. Short 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. AUC, area under the ROC.

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 (10–20%), it resulted in slightly higher rates of 30–40% 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 NUC includes periodical measurement of HBV DNA levels (real-time PCR) and ALT levels. This study revealed that

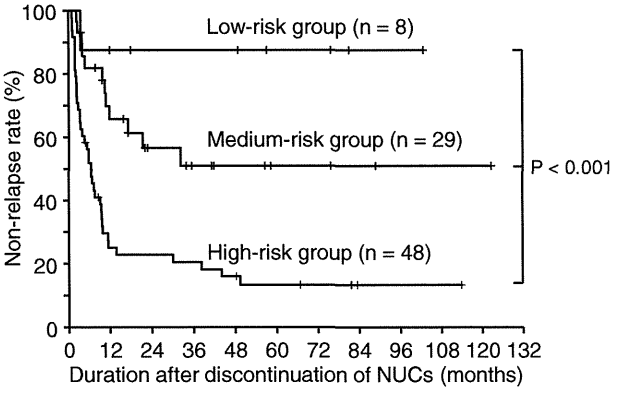

Figure 4 Comparison of non-relapse rates using the Kaplan–Meier method among three groups classified by the sum of the scores of hepatitis B surface antigen and HB core-related antigen levels at the time of nucleoside/nucleotide analog (NUC) discontinuation.

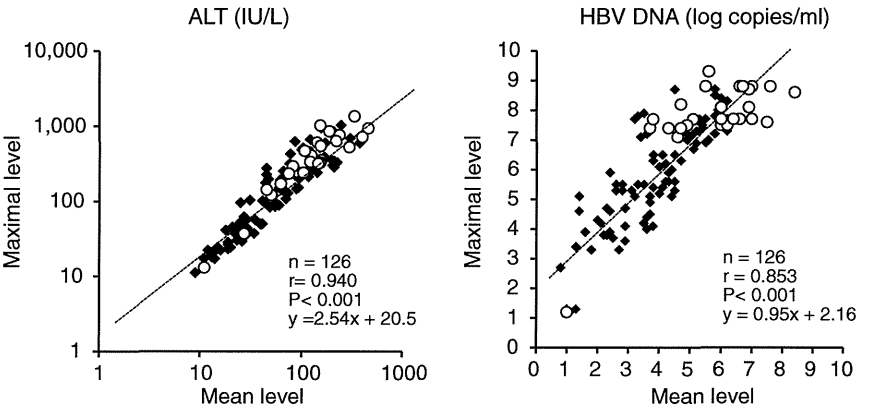

Figure 5 Correlation between maximal and mean levels of alanine aminotransferase (ALT) (left) and hepatitis B virus (HBV) DNA (right) after discontinuation of nucleoside/nucleotide analog (NUC). Open circles indicate patients with detectable hepatitis B e-antigen (HBeAg) and closed squares indicate patients without detectable HBeAg.

relapse after discontinuation occurs mostly within 1 year, gradually decreases after 1 year and rarely occurs after the first 3 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 2 weeks up to 16 weeks after discontinuation and every 4 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 approximately two-thirds patients who would finally achieve the inactive carrier state. Therefore, even if the ALT or HBV DNA levels show 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 NUC 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 (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/mL corresponded to the maximum HBV DNA of 5.7 log copies/mL. Patients with ALT values of not less than 80 IU/L after discontinuation are highly likely to show a mean value of more than 30 IU/L and not assumed to finally meet the criteria for successful discontinuation. Similarly, patients with HBV DNA value of not less than 5.8 log copies/mL after discontinuation are most likely to show a 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 ALT value of not less than 80 IU/L or 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 for retreatment with NUC. It is considered that NUC 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 also can 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 NUC. 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 Appendix 1-V. This guideline provides information to support physicians to decide NUC discontinuation timing but physicians should actually consider for each patient whether NUC 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 NUC cannot be successfully discontinued, one of the options is re-administration of NUC. However, it has not been investigated whether re-administration of NUC results in the emergence and development of resistant strains. Further, it is not resolved which NUC should be given when re-administration is required. The consent of 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 sensitivity HBV DNA, HBV RNA,[13,14] HBV genotypes and HBV genetic mutations. Because these guidelines were prepared based on retrospective studies, it is necessary to validate them with prospective studies. In addition, how to actively discontinue NUC by sequential treatment with interferon also should be included as an important issue to be investigated.

Three kinds of NUC are available now in Japan. Lamivudine was 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 leading to a better one in the future.

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APPENDIX

Guidelines for avoiding risks resulting from discontinuation of nucleoside/nucleotide analogs 2012

I. Aims of these guidelines

IN TREATMENT WITH nucleoside/nucleotide analogs (NUC) in patients with chronic hepatitis B, it is an important treatment goal to aim at drug-free status by discontinuation of NUC. However, discontinuation of NUC often results in hepatitis relapse which may become severe. Sufficient consideration must be given to the risk in case of discontinuation.

Hepatitis B surface antigen (HBsAg) negativity is the goal of treatment with NUC, but it cannot be always achieved easily. Therefore, discontinuation may be considered even if HBsAg remains positive. These guidelines aim to discontinue NUC in such conditions and finally achieve the inactive carrier state (alanine aminotransferase [ALT] <30 IU/L and hepatitis B virus [HBV] DNA level in blood <4.0 log copies/mL).

It is currently unknown which of the two options for NUC, 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 NUC 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 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. (NUC 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 nucleoside/nucleotide analogs.
   Almost all patients with high proliferative potential of HBV will relapse after discontinuation. It is essential not to discontinue NUC in these patients and the requirements were determined as follows: (i) HBV DNA level in blood is negative (real-time PCR) at the time of discontinuation; and (ii) hepatitis B e-antigen (HBeAg) level in blood is negative at the time of discontinuation.
2. Condition for duration of treatment period of NUC.
   Because short-term treatment with NUC can easily result in relapse, it is recommended to meet the following condition: more than 2 years after the initial administration of NUC.
3. Assessment of relapse risk by scoring of viral antigen levels.
   For the patients who meet the requirements for discontinuation (HBV DNA negative and HBeAg negative at the time of discontinuation), the HBsAg level and the HBcrAg 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 NUC should be discontinued or not by reference to it to reduce the relapse risk.