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B型肝炎核心關聯抗原在停止類核苷(酸)治療後的動態測量以預測後續臨床肝炎復發

Dynamic Measurement of Hepatitis B Core-related Antigen After Cessation of Nucleos(t)ide Analogues to Predict Subsequent Clinical Relapse

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**Dynamic Measurement of Hepatitis B Core-related Antigen After Cessation of Nucleos(t)ide Analogues to Predict Subsequent Clinical Relapse**

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**中文摘要**

**背景：**核苷(酸)類似物固定療程被提議作為慢性B型肝炎的治療策略，但監測治療後反應的生物標誌物仍很有限。

**目的：**我們研究了治療後測量B型肝炎核心相關抗原(HBcrAg)的動態測量是否可以預測後續臨床復發風險。

**方法：**此為多中心回溯研究，分析納入了停用entecavir或tenofovir後接受前瞻性監測的慢性B型肝炎病人，治療結束時(EOT)HBeAg需為陰性且HBV DNA不可檢測，並排除肝硬化或惡性腫瘤患者。臨床復發的定義是血清丙氨酸轉氨酶(ALT) 超過正常上限兩倍且再次出現病毒血症。我們應用了時間依賴的Cox比例風險模型來釐清HBcrAg水平和後續臨床復發的關聯。

**結果：**本研究包括203名患者(中位數年齡，49.8歲；76.8%男性；60.1%使用entecavir)，治療的中位數為36.9個月(四分位數範圍[IQR]，36.5-40.1)。在中位數治療後的隨訪31.7個月(IQR, 16.7-67.1)中，104名患者出現CR，5年累積發生率為54.8%(95%信心區間[CI]，47.1% - 62.4%)。在調整了EOT HBsAg、anti-HBe和EOT ALT的多變量模型中，時間變化的HBcrAg水平是臨床復發的顯著危險因子(風險比[aHR]，每log U/mL 1.43；95% CI，1.16-1.76)。追蹤期間，HBcrAg < 1,000 U/mL預測較低的臨床復發風險(aHR, 0.31；95% CI, 0.17-0.57)。

**結論：**停止核苷(酸)類似物治療後的HBcrAg動態測量可以預測後續的臨床復發。

**關鍵詞：**慢性B型肝炎；有限的核苷(酸)類似物治療；乙型肝炎核心相關抗原。

**ABSTRACT**

**Background:** Finite nucleos(t)ide analog (NA) therapy has been proposed as an alternative treatment strategy for chronic hepatitis B (CHB), but biomarkers for post-treatment monitoring are limited.

**Aim:** We aimed to examine if dynamic measurement of serum HBcrAg levels after cessation of NA therapy is associated with risk of clinical relapse.

**Methods:** This retrospective multicenter analysis enrolled adults with CHB who were prospectively monitored after discontinuing entecavir or tenofovir with negative HBeAg and undetectable HBV DNA at the end of treatment (EOT). Patients with cirrhosis or malignancy were excluded. CR was defined as serum alanine aminotransferase (ALT) > two times the upper limit of normal with recurrent viremia. We applied time-dependent Cox proportional hazard models to clarify the association between HBcrAg levels and subsequent CR.

**Results:** The cohort included 203 patients (median age, 49.8 years; 76.8% male; 60.1% entecavir) who had been treated for a median of 36.9 months (interquartile range [IQR], 36.5-40.1). During a median post-treatment follow-up of 31.7 months (IQR, 16.7-67.1), CR occurred in 104 patients with a 5-year cumulative incidence of 54.8% (95% confidence interval [CI], 47.1% ‐ 62.4%). Time-varying HBcrAg level was a significant risk factor for subsequent CR (adjusted hazard ratio [aHR], 1.43 per log U/mL; 95% CI, 1.16-1.76) in the multivariable model adjusted for EOT HBsAg, anti-HBe, and EOT ALT. During follow-up, HBcrAg < 1,000 U/mL predicted a low risk of CR (aHR, 0.31; 95% CI, 0.17-0.57).

**Conclusions:** Dynamic measurement of HBcrAg after NA cessation is predictive of subsequent CR and may be useful to guide post-treatment monitoring.

**Keywords:** chronic hepatitis B; finite nucleos(t)ide analog therapy; hepatitis B core-related antigen

**Introduction**

Nucleos(t)ide analogs (NA) suppresses the replication of HBV by inhibiting the viral reverse transcriptase, thereby reducing hepatic inflammation, other liver-related comorbidities and hepatocellular carcinoma (HCC).(1-3) However, the ideal treatment endpoint, i.e., hepatitis B surface antigen (HBsAg) seroclearance, is rarely achieved during NA treatment.(4, 5) Most patients who remained HBsAg-positive experienced viral relapse after stopping NA. Hepatitis flares can occur following viral relapse, and severe acute exacerbations can lead to fatal consequences in some patients.(6, 7) Nevertheless, a limited number of studies have reported high HBsAg seroclearance rates after cessation of NA(8, 9) and ex vivo studies also indicate that HBV-specific T cell responses could be enhanced by discontinuing treatment with viral suppressants.(10, 11) Thus, identification of groups of patients with chronic hepatitis B (CHB) who need to maintain NA or who can safely discontinue NA is an important yet unresolved issue.

HBcrAg is a potentially novel and useful biomarker that includes the hepatitis B core antigen (HBcAg), hepatitis B e antigen (HBeAg), and a truncated precore protein (p22Cr).(12) Serum HBcrAg level correlates with nuclear cccDNA activity, and could be used to predict HBeAg seroconversion, sustained treatment response to NA, and the risk of development or recurrence of HCC.(13-16) Prior studies have demonstrated that serum HBcrAg level at the time of NA cessation is useful for predicting off-therapy relapse and identifying patients for whom NA discontinuation may be potentially suitable.(17-19) However, the levels of HBcrAg can change after cessation of treatment, and it is unclear whether dynamic measurements of HBcrAg off-therapy may better predict subsequent CR than the fixed HBcrAg level measured at EOT. Moreover, the relationship between serial changes in HBcrAg after NA cessation and the risk of relapse remains unknown. Thus, this study retrospectively used data and biospecimens collected from a multicenter, prospectively enrolled patient cohort to serially measure HBcrAg levels off therapy and to clarify the associations between dynamic off-therapy HBcrAg levels and subsequent CR after NA discontinuation.

**Hypothesis**

Dynamic measurement of serum HBcrAg levels after cessation of NA therapy is associated with risk of clinical relapse

**Primary aim**

To clarify the association between dynamics of HBcrAg level and relapse risk

**Secondary aims**

- To elucidate the association between serial changes in HBcrAg level and relapse risk

- To investigate the predictive performance of HBcrAg level <1,000 U/mL as the cutoff

**Methods and Materials**

***Design and setting***

This was a retrospective analysis based on a prospective multicenter cohort study conducted in three teaching hospitals (E-Da Hospital, Kaohsiung, Lotung Poh-Ai Hospital, Yilan, and National Taiwan University Hospital Yun-Lin Branch, Yunlin) in Taiwan. Adult (age > 20 years) CHB patients without cirrhosis who discontinued NA therapy using either entecavir or tenofovir were assessed for eligibility.

***Study participants***

In the initial cohort, adult patients with CHB who were going to discontinue NA therapy between July 1, 2011 and April 1, 2015 were screened. Patients were included if they had been diagnosed with CHB for at least 6 months prior to NA treatment, continuously received any NA (lamivudine, adefovir, telbivudine, entecavir, or tenofovir) for at least 3 years, were serologically negative for HBeAg, and showed undetectable levels of HBV DNA at the end of NA therapy. Patients were excluded in the presence of coinfection with human immunodeficiency virus or hepatitis C virus, any malignancy, liver cirrhosis, hepatic encephalopathy, variceal hemorrhage, organ transplantation, previous use of interferon alpha for 1 month or longer, and concurrent use of cytotoxic or immunosuppressive medication. The diagnosis of liver cirrhosis was based on pathological proof or clinical criteria that included splenomegaly or esophagogastric varices in addition to typical sonographic features.

In the current study, we only include patients who discontinued NA therapy using either entecavir or tenofovir. Eligible patients had been treated for at least 2 years with HBeAg-serology confirmed to be negative and serum HBV DNA undetectable at treatment cessation.

***Follow-up after treatment cessation***

Pertinent demographic, biochemical, serological, and virological data were collected at enrollment. After discontinuation of NAs, patients were monitored at a close interval of 3 months. The patients underwent physical checkup and laboratory measurement at each follow-up visit. They also underwent abdominal sonography along with serum alpha-fetoprotein estimation tests every 6 months for the surveillance of liver cancer.

Serum levels of HBcrAg were quantitatively analyzed upon the end of treatment, one year, and two years after treatment cessation, using a commercialized kit (Lumipulse HBcrAg; Fuji-Rebio, Tokyo, Japan). Serum HBsAg levels were measured through an automated immunoassay (Abbott Architect i2000, Abbott Park, IL, USA). Samples with HBsAg levels exceeding the upper limit of automatic detection (250 IU/mL) were manually diluted before quantification. Serum HBV DNA was quantified through a commercialized polymerase chain reaction method (COBAS TaqMan HBV Test, version 2.0, Roche Molecular Systems, Inc., Branchburg, NJ, USA) with a detection range of 20–1.7 × 108 IU/mL.

***Definitions of the study endpoints***

The primary study outcome was clinical relapse and was defined as an episode of elevated ALT (>80 IU/mL, >2 times the normal conventional upper limit) and >2000 IU/mL HBV DNA. Patients did not resume antiviral therapy until clinical hepatitis persisted for 3 months or longer, unless a risk of hepatic decompensation (serum bilirubin >2 mg/dL or prothrombin time prolonged >3 seconds) was observed.

***Statistical analysis***

Continuous and categorical variables were summarized using the median and interquartile range (IQR) and proportion with exact numbers, respectively. The incidence rates of virological and clinical relapses were estimated using the Kaplan–Meier method. In a multivariate-adjusted Cox proportional hazards model for off-therapy relapses, the serum level of HBcrAg was a time-varying variable that denoted each measurement after NA cessation. The dose–response relationship for the association between HBcrAg levels and off-therapy relapses was illustrated by penalized splines in the Cox model. The results were reported as hazard ratios along with 95% confidence intervals (CIs). Data were analyzed using commercial software (Stata, version 13.0; Stata Corp, College Station, TX, USA). All statistical analyses were two-sided with significance set at P < 0.05.

**Results**

***Baseline characteristics of the study participants***

This study included 156 male (76.8%) and 47 female (23.2%) patients with CHB who discontinued NA, with the median age of 49.8 years (IQR, 41.9, 59.0). Most patients (*n* = 123, 60.6%) received entecavir and the median duration of treatment was 36.9 months (IQR, 36.5, 40.1). The EOT serum HBsAg was 2.7 (IQR, 2.0, 3.0) log IU/mL and the EOT serum HBcrAg was 3.0 (IQR, 2.0, 3.9) log U/mL, respectively. The median follow‐up time was 31.7 months (IQR, 16.7, 67.1) (**Table 1**).

***CR after discontinuation of NA and association with EOT HBcrAg level***

During the follow‐up period, CR occurred in 104 patients with a cumulative incidence of 49.5% (95% CI, 42.2% ‐ 56.7%), 54.8% (95% CI, 47.1% ‐ 62.4%) and 58.2% (95% CI, 50.1%‐66.0%) at 3, 5, and 7 years, respectively (**Figure 1**). Eight patients experienced hyperbilirubinemia with serum total bilirubin over 2 mg/dL after CR, but recovered fully after resumption of NA therapy.

The lower limit of HBcrAg detection in the current study was 1,000 U/mL. This value was used as the cutoff as suggested by the Japan Society of Hepatology (JSH) (22). The incidence of CR was significantly higher with the EOT HBcrAg ≥ 1,000 U/mL than <1,000 U/mL (*P* = 0.002) (**Figure 2**). In the univariable analysis, the HR for CR was 1.30 (95% CI, 1.10‐1.53) per log U/mL (*P* = 0.005) for the EOT HBcrAg (**Table 2**). In the receiver operating characteristic curve designed to evaluate the performance of EOT HBcrAg, however, the EOT HBcrAg level alone could not sufficiently predict the risk of CR, with an AUC of 0.61 (95% CI, 0.53‐0.69) (**Figure 3**).

***Changes in serum HBcrAg in post-treatment monitoring and the associations with CR***

After stopping treatment, 114 and 71 patients remained off NA after the first and second years of follow-up. Their posttreatment fluctuations in HBcrAg levels were illustrated in relation to the occurrence of CR (Figure 4). In patients with CR and those without CR, the median HBcrAg levels were 3.20 log U/mL (n=104) vs. 2.00 log U/mL (n=99) at treatment cessation (*P* =0.004), 2.00 log U/mL (n=35) vs. 2.00 log U/mL (n=79) at year one (*P* = 0.191), and 3.54 log U/mL (n=14) vs. 2.00 log U/mL (n=57) at year two (*P* = 0.006).

We further examined the association between the pattern of HBcrAg changes and the risk of CR. Among the 114 patients who did not resume antiviral therapy at year one, serum HBcrAg decreased in 39 patients (34.2%), did not change in 62 patients (54.4%) and increased in 13 patients (11.4%). The incidence of subsequent CR did not differ between patients with and without decrease in serum HBcrAg inf the first year (*P* = 0.74; **Figure 5A**). During the second year, serum HBcrAg decreased in 16 patients (22.5%), did not change in 41 patients (57.8%), and increased in 14 patients (19.7%). Similarly, there were no difference in the risk of CR between patients with and those without HBcrAg decreases in year two (*P* = 0.84; **Figure 5B**).

***Multivariable Cox proportional hazard analysis for time-varying HBcrAg levels***

In the multivariable Cox proportional hazard analysis, the time-varying HBcrAg level and the EOT HBcrAg level were examined as either fixed factors or not. After stepwise selection, independent predictors of subsequent CR included EOT anti-HBe antibody positivity (adjusted hazard ratio [aHR], 0.34; 95% CI, 0.21-0.56), EOT HBsAg (aHR, 1.39 per log IU/mL; 95% CI, 1.02-1.90), time-varying HBcrAg level (aHR, 1.33 per log U/mL; 95% CI, 1.13-1.58) and EOT ALT (aHR, 1.01 per U/L; 95% CI, 1.00-1.02) (**Table 2**).

When the time-varying HBcrAg level and the EOT HBcrAg level were forced into the model (**Supplementary Table 1**), it was the time-varying level (aHR, 1.43 per log U/mL; 95% CI, 1.16-1.76), instead of the fixed EOT level (aHR, 0.78 per log U/L; 95% CI, 0.56-1.11), that was significantly associated with the risk of CR.

***HBcrAg level <1,000 U/mL as the cutoff to stratify the risk of CR off NA therapy***

At treatment cessation, 98 patients had a serum level of HBcrAg <1,000 U/mL and 19 patients (19.4%) in this group developed CR. Conversely, CR occurred in 45 of 105 (42.9%) patients with HBcrAg >1,000 U/mL. Among 114 patients who remained in the cohort without antiviral retreatment during the first year, 11 of 72 (15.3%) patients with HBcrAg <1,000 U/L, and 7 of 42 (16.7%) patients with HBcrAg ≥1,000 U/mL, suffered CR, respectively. In the second year, CR occurred in 3 of 44 (6.8%) and 4 of 27 (14.8%) patients with HBcrAg < and ≥1,000 U/mL, respectively.

HBcrAg < or ≥1,000 U/mL was analyzed as a time-varying variable in the multivariable Cox proportional hazard model. Serum HBcrAg <1,000 U/mL predicted a lower risk of off-therapy CR with an aHR of 0.31(95% CI, 0.17-0.57) (**Table 3**). When dynamic HBcrAg was compared against EOT HBcrAg, the time-varying variable again outperformed the fixed level in the association with subsequent CR in the adjusted analysis (aHR, 0.30; 95% CI, 0.13-0.70) (**Supplementary Table 2**).

**DISCUSSION**

In this multicenter cohort study of adult patients with CHB prospectively followed up after cessation of NA, we found that the dynamic measurement of HBcrAg as a time-varying predictor outperformed the static measurement at treatment cessation to stratify the risk of subsequent CR. However, the change in serum HBcrAg level was generally mild after cessation of NA and the pattern of changes (i.e., with or without decrease during the predicting year) was not independently associated with CR. We further showed that risk stratification could be achieved by HBcrAg < or ≥ 1,000 U/mL, a convenient cutoff that can be easily applied in daily practice. These findings implicate that dynamic measurement of serum HBcrAg may inform post-treatment monitoring in CHB patients who stop NA therapy.

Identification of patients who can safely withdraw from NA is crucial for the practice of finite NA strategy, and there is an unmet need for accurate and applicable biomarkers. Previous studies have shown that lower HBsAg or HBcrAg level at EOT were associated with lower rates of relapse after discontinuation of NA.(17, 20) Sonneveld et al. enrolled 572 Asian and European patients with a median NA treatment duration of 295 weeks in a multicenter study and reported that a lower EOT HBcrAg level was related to better outcomes after cessation of NA, including sustained virologic response, HBsAg loss, and a lower ALT flare rate.(23) Other studies suggested that a combination of the EOT HBcrAg level with either HBV RNA or HBsAg level at EOT may be an acceptable predictor of off-therapy relapse.(19, 24, 25) In the present study, we also found that the incidence of CR was significantly lower among patients with HBcrAg < 1,000 U/mL at EOT. Moreover, the EOT HBcrAg level was useful for stratifying the risk of CR after discontinuation of NA. Therefore, a growing body of data support that both EOT HBsAg and EOT HBcrAg levels can help to assess the risk of clinical hepatitis following NA withdrawal. However, EOT HBcrAg level alone is not accurate enough to predict CR, as indicated by its AUC of 0.61 (95% CI, 0.53‐0.69) in our study.

After NA cessation, the serum levels of HBsAg and HBcrAg may change over time, and it is far less clear whether dynamic measurements may add to the accuracy of prediction for subsequent CR. In our prior study with 140 patients, we reported that the time-varying gradient of serum HBsAg was associated with both clinical and virological relapse.(26) In the present study, we demonstrated for the first time that serum HBcrAg could fluctuate after NA withdrawal and, more importantly, the time-varying level of serum HBcrAg was more accurate than the static EOT level to predict the risk of CR. These findings referred to that the most recent measurement of serum HBcrAg significantly outperformed the EOT level in predicting CR among patients who had not yet experienced a relapse **(Supplementary Figure 1**) and may have important implications in clinical care of patients who stop NA therapy. Currently, only serum HBV DNA and ALT levels are recommended in guidelines for post-treatment monitoring. (27) Though these two biomarkers are crucial, they usually disclose an imminent event rather than provide an early forecast. A fierce surge in viremia usually predicts an acute HBV flare-up is near, and elevation of serum ALT is actually a marker of liver injury that has already occurred. Based on our finding, post-treatment dynamic measurements of HBsAg and HBcrAg may complement HBV DNA and ALT to monitor those who discontinue NA.

While serum HBcrAg levels may change over time following NA cessation, we found that the fluctuation was generally mild during the first and second years off-NA therapy. In fact, the serum level of HBcrAg was stable without marked increase or decrease in most patients. Accordingly, it seemed reasonable that the pattern of changes in serum HBcrAg was not significantly associated with CR, because the interval difference was mild for most patients. This finding suggested that cessation of NA therapy did not significantly affect the transcriptional activity of HBV cccDNA but further research, preferably with quantitative analysis of viral expression in the hepatocytes, is needed to fully understand the biological underpinning.

The strengths of our study are as follows: First, HBcrAg kinetics during the off-therapy period were assessed using serum HBcrAg measurements regularly obtained three times according to a prespecified protocol. Second, the participants were prospectively enrolled from multiple sites and the concern of selection bias commonly observed in retrospective analyses and/or single-center experience could be mitigated. Third, patients who took either entecavir or tenofovir were included, which reflects the current standard of care. Although the patterns of relapse for patients on these two regimens differ,(28) the design of this study more closely reflects daily clinical practice. Fourth, the follow-up period was sufficiently long enough to observe the outcomes after cessation of NA. Even though the incidence of hepatitis flares was high, and many patients resumed NA and discontinued observation, more than one-third of the patients (*n* = 74, 36.5%) in this cohort remained at risk of CR after 3 years of follow‐up. Finally, our multivariable Cox proportional hazard analysis was developed with the adjustment for EOT HBsAg level and EOT HBcrAg level that were important factors associated with subsequent CR after NA cessation.

There are several limitations to this study. First, due to the health insurance policy in Taiwan, the present study enrolled patients with a median treatment duration of 36.9 months (IQR, 36.5-40.1). (2) The small range of the treatment duration may lead to selection bias, and we could not examine the association between the duration and outcome of treatment. Second, we could not precisely quantify serum HBcrAg levels when HBcrAg levels were lower than 1,000 U/mL. However, the same commercial assay was employed in several prior studies, and is widely used in the clinic. (29) Third, this cohort only included Asian patients and the most common HBV genotypes were type B or C. (29) Finally, we could not exactly investigate the association between the relapse risks and the different patterns of patients with non-decreased HBcrAg level because of the limited number of patients remained untreated. Therefore, future studies of more participants with different ethnicities, HBV genotypes and NA treatment durations are required to validate our findings.

In conclusion, this study revealed that the dynamic measurements of serum HBcrAg after cessation of NA outperformed the EOT HBcrAg level as an independent risk factor for subsequent CR. HBcrAg level < 1,000 U/mL could be a useful cutoff value to forecast a low risk of subsequent CR during the off-therapy follow-up. These finding could help to design a safer monitoring strategy for patients who discontinue NA and may inspire further research to optimize the finite strategy of NA therapy.

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**Table 1**. Baseline characteristics of the study patients

|  |  |
| --- | --- |
| Characteristics† | All patients (*N* = 203) |
| Age, years | 49.8 (41.9, 59.0) |
| Male gender, n (%) | 156 (76.8) |
| Positive anti-HBe, n (%) | 192 (94.6) |
| HBsAg, log IU/mL | 2.7 (2.0, 3.0) |
| HBcrAg, log U/mL | 3.0 (2.0, 3.9) |
| AST, IU/L | 27 (23, 35) |
| ALT, IU/L | 27 (19, 40) |
| Anti‐viral regimen |  |
| Entecavir, n (%) | 123 (60.6) |
| Tenofovir, n (%) | 80 (39.4) |
| Duration on therapy, months | 36.9 (36.5, 40.1) |
| Duration of follow‐up, months | 31.7 (16.7, 67.1) |
| Pre‐treatment positive HBeAg, n (%) | 32 (15.8) |
| Pre‐treatment positive anti‐HBe, n (%) | 168 (82.8) |
| Pre‐treatment AST, U/L | 68 (40, 123) |
| Pre‐treatment ALT, U/L | 103 (54, 212) |

ALT, alanine transaminase; anti‐HBe, hepatitis B e antibody; AST, aspartate transaminase; HBcrAg, hepatitis B core‐related antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen.

†Status at the cessation of anti‐viral therapy unless otherwise specified;

values expressed as percentage or median [IQR].

**Table 2.** Multivariable Cox proportional hazard model for the risk of clinical relapse

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Univariable analysis | | Multivariable analysis | | |
| Variables | HR | 95% CI | Adjusted HR | 95% CI | *P*-value |
| Age, years | 1.00 | 0.98-1.02 |  |  |  |
| Male sex | 2.16 | 1.25-3.73 |  |  |  |
| EOT positive anti‐HBe | 0.71 | 0.35-1.46 | 0.34 | 0.21-0.56 | <0.001 |
| EOT HBsAg, log IU/mL | 1.84 | 1.47-2.30 | 1.39 | 1.02-1.90 | 0.036 |
| EOT HBcrAg, log U/mL | 1.30 | 1.10-1.53 |  |  |  |
| Time-varying HBcrAg level, log U/mL | 1.36 | 1.14-1.63 | 1.33 | 1.13-1.58 | <0.001 |
| EOT ALT, U/L | 1.00 | 1.00-1.01 | 1.01 | 1.00-1.02 | <0.001 |
| Tenofovir use (vs entecavir) | 0.80 | 0.54-1.18 |  |  |  |
| Duration on therapy, months | 0.99 | 0.97-1.00 |  |  |  |
| Pre‐treatment positive HBeAg | 1.29 | 0.80-2.08 |  |  |  |
| Pre‐treatment positive anti‐HBe | 0.83 | 0.52-1.32 |  |  |  |

The measurements were conducted at the end of treatment if not otherwise specified. ALT, Alanine transaminase; anti‐HBe, hepatitis B e antibody; HBcrAg, hepatitis B core‐related antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen.; HR, hazard ratio

**Table 3**. Multivariable Cox proportional hazard model for the risk of clinical relapse and HBcrAg as a time-varying and categorical variable

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Univariable analysis | | Multivariable analysis | | |
| Variables | HR | 95% CI | Adjusted HR | 95% CI | *P*-value |
| Age, years | 1.00 | 0.98-1.02 |  |  |  |
| Male sex | 2.16 | 1.25-3.73 | 2.63 | 1.13-6.13 | 0.025 |
| EOT positive anti‐HBe | 0.71 | 0.35-1.46 |  |  |  |
| EOT HBsAg, log IU/mL | 1.84 | 1.47-2.30 |  |  |  |
| EOT HBcrAg, log U/mL | 1.30 | 1.10-1.53 |  |  |  |
| HBcrAg < 1000 U/mL (vs ≥ 1000 U/mL)† | 0.33 | 0.18-0.92 | 0.31 | 0.17-0.57 | <0.001 |
| EOT ALT, U/L | 1.00 | 1.00-1.01 |  |  |  |
| Tenofovir use (vs entecavir) | 0.80 | 0.54-1.18 |  |  |  |
| Duration on therapy, months | 0.99 | 0.97-1.00 |  |  |  |
| Pre‐treatment positive HBeAg | 1.29 | 0.80-2.08 |  |  |  |
| Pre‐treatment positive anti‐HBe | 0.83 | 0.52-1.32 |  |  |  |

The measurements were conducted at the end of treatment if not otherwise specified. ALT, Alanine transaminase; anti‐HBe, hepatitis B e antibody; HBcrAg, hepatitis B core‐related antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen.; HR, hazard ratio. †As a time varying variable

**FIGURES**



**Figure 1.** The cumulative incidence of clinical relapse following discontinuation of nucleos(t)ide analogues in the study population.



**Figure 2.** The cumulative incidence of clinical relapse according to serum level of hepatitis B core-related antigen measured at the end of treatment.

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**Figure 3.** The receiver operating characteristic curve of serum end-of-treatment hepatitis B core-related antigen level to predict clinical relapse off nucleos(t)ide analogues.



**Figure 4.** The violin plot illustrates the distribution of serum HBcrAg levels measured at treatment cessation (left panel), one year afterwards (middle panel), and two years afterwards (right panel) in all patients, categorized by the occurrence (indicated in yellow) or absence (indicated in blue) of clinical relapse.



**Figure 5.** The cumulative incidence of clinical relapse according to changes in serum level of hepatitis B core-related antigen during the first year (5A, left panel) and second year (5B, right panel)