財團法人明日醫學基金會研究計畫申請書

計畫名稱	（中文）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	
	（英文）Dynamic Measurement of Hepatitis B Core-related Antigen After Cessation of Nucleos(t)ide Analogues to Predict Subsequent Clinical Relapse	
計畫類別	☑個別型	☐整合型
計畫歸屬	☐基礎醫學☐生物醫學☑臨床醫學☐資訊系統☐醫院管理☐整合性醫學研究	
計畫主持人	姓名：許耀峻 Yao-Chun Hsu	機關：**義大醫院**
	職稱：**中心主任**	單位：**肝病中心**
通訊地址	**高雄市左營區重化街六號 13 樓**	
聯絡電話	**(02)6150011, 0988687726**	
計畫執行期限	自 **111** 年 1 月 **1** 日起至 **111** 年 **12** 月 **31** 日止	
共同主持人（一）	姓名：	機關：
	職稱：	單位：
共同主持人（二）	姓名：	機關：
	職稱：	單位：
共同主持人（三）	姓名：	機關：
	職稱：	單位：
共同主持人（四）	姓名：	機關：
	職稱：	單位：

研究計畫摘要

研究主題: 在慢性 B 型肝炎患者停止抗病毒治療後動態測量 B 型肝炎核心關聯抗原以預測停藥後臨床肝炎復發的風險

一、試驗目的：

1. 驗證 B 型肝炎核心關聯抗原可否作為準確的生物標誌已預測停止類核苷(酸)藥物治療後肝炎復發的風險

二、研究背景：

我們以往的研究已發現，慢性 B 型肝炎患者在停止核苷（酸）類似物時所測量的 B 型肝炎核心關聯抗原（HBcrAg）血清濃度，和停藥後慢性 B 型肝炎的復發風險顯著相關。然而，HBcrAg 的血清濃度可能會在治療停止後發生變化，目前尚不清楚這個生物標誌在停藥後的血清濃度變化與復發風險的關係。本研究擬釐清停藥後的動態測量相較於治療結束當下的單點測量，是否更能準確預測隨後的臨床復發。

三、研究方法：

本研究建立在此前一項前瞻性多中心世代研究，將回溯分析病人資料與血清檢體。該世代研究納入成人（年齡 ＞20 歲）慢性 B 型肝炎且無肝硬化的患者。符合條件的患者須已經接受了至少 2 年的恩替卡韋(entecavir)或替諾福韋(tenofovir disoproxil fumarate)治療後停藥，B 肝 e 抗原(HBeAg) 血清學檢查證實為陰性，並且在停止治療時血清無法檢測到 HBV DNA。我們將檢測患者在治療結束時、停藥一年後，和停藥兩年後血清中 HBcrAg 的濃度。主要研究結果是臨床復發，定義為血清丙氨酸氨基轉移酶 (ALT) 檢驗值超過正常上限的兩倍（常規上限為 40 U/L）。我們將使用 Cox 比例風險模型以探討與臨床肝炎復發相關的危險因子，其中 HBcrAg 血清濃度的動態測量，將作為隨時間改變的自變項。

關鍵詞:慢性 B 性肝炎；B 型肝炎核心關聯抗原；抗病毒治療；停藥後復發

研究計畫目的及背景說明

Chronic infection with hepatitis B virus (HBV) is the leading cause of liver-related morbidity and mortality worldwide, particularly in Asian countries [1]. Management of patients with chronic hepatitis B (CHB) has reached an era of antiviral therapy, with approved regimens consisting of interferon alpha and nucleos(t)ide analogs (NAs) [2-5]. Through the effective inhibition of viral replication, NAs not only ameliorate viremia and reduce hepatic inflammation, but also may prevent and even reverse liver fibrosis [6-8]. A large body of evidence corroborates the effectiveness of NAs in improving clinical outcomes [9,10]. However, off-therapy durability after discontinuation of NA treatment is typically unsustainable [11-19].

Because of high off-therapy relapse rates, major international guidelines currently recommend an indefinite prolongation of NA therapy, possibly until loss of hepatitis B surface antigen (HBsAg) with or without appearance of accompanying antibodies [12]. However, this strategy entails life-long treatment for most treated patients [13]and is not affordable in regions with resource-constrained health care systems, where, ironically, CHB is most prevalent [14]. Recently, intense research has been carried out to clarify predictors of off-therapy relapse and identify patients who maintain remission without resuming medication [17-22].

In response to the challenges associated with the safe discontinuation of NAs, we prospectively followed a multicenter cohort who discontinued NAs after a minimum of 3 years on therapy. We observed that the serum level of hepatitis core-related antigen HBcrAg measured at the end of treatment (EOT) was able to stratify the risk of clinical relapses [20]. The level of HBsAg, however, may change over time after the cessation of NA therapy, but its clinical implication has not been elucidated. Therefore, we

conducted this study to explore the association between relapse risks and time-varying serum gradients of HBcrAg measured during an off-therapy follow-up.

Hypothesis

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

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 $P < 0.05$.

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