**財團法人明日醫學基金會研究計畫申請書**

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| 計畫名稱 | （中文）慢性B型肝炎患者接受抗病毒藥物後Mac-2結合蛋白糖基化異構體血清濃度的變化與後續肝癌風險的關係 | |
| （英文）Serial changes in serum M2BPGi level and risk of hepatocellular carcinoma after antiviral therapy in chronic hepatitis B | |
| 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 | |
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**研究計畫摘要**

**研究主題:** 探討Mac-2結合蛋白糖基化異構體的血清濃度在慢性B型肝炎患者抗病毒療程中的變化與預測肝癌風險的應用

**ㄧ、試驗目的：**

1. 釐清慢性B型肝炎患者服用抗病毒藥物後Mac-2結合蛋白糖基化異構體血清濃度隨時間的改變

2. 檢視Mac-2結合蛋白糖基化異構體血清濃度在預測肝癌風險的價值

**二、研究背景：**

慢性B型肝炎是全世界的重要公共衛生問題，是國人罹患肝癌的主要病因，雖然類核苷(酸)口服抗病毒藥物可以有效抑制病毒複製，減少肝癌發生風險，但是仍有病人在抗病毒治療後罹患肝癌，如何準確預測肝癌風險仍尚待解決。

Mac-2結合蛋白糖基化異構體（M2BPGi）是肝臟疾病的新興生物標誌，既往研究顯示與肝纖維化程度有關，也有相關文獻指出可用於肝癌風險預測。但是，應用於慢性B型肝炎患者的文獻仍然非常缺乏，且沒有長期的世代研究。

**三、研究方法：**

　　本計畫為多中心世代研究，將與美國史丹佛大學與日本名古屋市立大學合作，分析義大醫院和高雄醫學大學附設醫院的384位先前未接受抗病毒治療的慢性B型肝炎患者。患者在療程開始前與療程中連續收集血清，包括用藥前與治療後的第1和2年。我們將定量血清中M2BPGi的濃度，分析在不同時間點的變化，並釐清和肝硬化的關係，接著探討不同時間點的血清濃度與後續肝癌發生的風險，以考克斯(Cox)比例風險模型來評估M2BPGi與肝癌風險之間的關聯，並建立預測模型。

**關鍵詞:**慢性B性肝炎；Mac-2結合蛋白糖基化異構體; 肝癌；類核苷酸藥物；風險預測

**研究計畫目的及背景說明**

Mac-2 binding protein (M2BP) is a glycoprotein involved in intercellular adhesion and interactions with the extracellular matrix 1. M2BP is widely expressed in various human tissues, but a liver-specific glycosylation isomer (M2BPGi) can be determined and quantified using the *Wisteria floribunda* agglutinin immunoassay that is already commercially available in Japan 2. In the recent years, M2BPGi has emerged as a novel biomarker that correlates well with hepatic fibrosis in patients with chronic liver diseases 3. Recent literature also suggests that M2BPGi may correlate with hepatocellular carcinoma (HCC) development in patients with chronic viral hepatitis 4-8, though most have been conducted in patients with chronic hepatitis C (CHC) or untreated chronic hepatitis B (CHB) without serial measurements.

Indeed, the serum concentration of M2BPGi is not static and may change in a relatively short period of time, especially in the setting of antiviral therapies. This was well described by Nagata and colleagues in their recent study of CHC patients treated with either interferon-based or interferon-free direct acting antiviral therapies 9. However, data on the longitudinal effect of antiviral therapy on M2BPGi levels in treated CHB patients is still limited. While CHB patients treated with NA can expect significantly reduced risk of HCC, this risk remains, especially in patients with cirrhosis 10-12. Therefore, it is particularly important to evaluate the dynamic changes and predictive potential of novel biomarkers such as M2BPGi longitudinally and in relation to antiviral therapy because levels can change with treatment, treated patients may still develop HCC, and long-term suppressive treatment with nucleos(t)ide analogues (NAs) remains the primary therapeutic strategy in the management of active CHB 13-15. Moreover, a convenient and inexpensive serum marker to accurately estimate HCC risk remains unavailable for CHB patients treated with NAs.

In order to address the aforementioned gaps in the current literature, we conducted this multicenter cohort study of treatment-naïve patients who had pretreatment and serial blood collection after the initiation of NA therapies to evaluate the association of M2BPGi levels with future HCC development. In addition, to accurately estimate this risk, we used time-dependent analytic methods and performed stratified analyses by cirrhosis status, given that M2BPGi is well-known to strongly correlate with liver fibrosis/cirrhosis.

**研究方法及步驟：**

***Study design and setting***

This cohort study included an exposed cohort of CHB patients with serum samples prospectively collected prior to NA therapy (baseline sample) and serially at year 1 and year 2 after treatment initiation and an unexposed control cohort of CHB patients who did not receive NA therapy during study period. Patients were enrolled and observed at 2 teaching hospitals in Kaohsiung, Taiwan (Kaohsiung Medical University Hospital and E-Da Hospital) between end of January 2000 and end of September 2017. All patients gave written informed consents. The laboratory analysis for the serum samples was conducted at a single laboratory at the Department of Virology and Liver unit, Nagoya City University, Nagoya, Japan. Clinical and laboratory data were submitted to the data center at Stanford University Medical Center, Palo Alto, California, USA for data management and analysis. This study was approved by the institutional review board at each participating institution.

***Patient population***

Patients were eligible if they were 18 years or older, had CHB (positive hepatitis B virus [HBV] surface antigen [HBsAg] or HBV DNA and a documented history of chronic infection for 6 months or longer), were treatment-naïve at the time of the first serum collection (baseline level) and were subsequently treated with a commercially approved NA and had serum collection again at 1 year and/or 2 years after NA therapy initiation. Subjects with co-infection with hepatitis C virus or another cause of chronic liver disease, or any malignancy at the time of NA initiation were excluded. Those who developed HCC within one year of therapy were also excluded as HCC in this case may be prevalent instead of incident cases.

The indications of antiviral therapy principally followed the practice guidelines endorsed by the Asian Pacific Association for the Study of the Liver.14 In general, the manifestation of hepatic decompensation, severity of liver fibrosis, status of hepatitis B e antigen (HBeAg), serum levels of HBV DNA and alanine aminotransferase (ALT) were all taken into consideration.

The selection of untreated control patients followed the same eligibility criteria as described above, except for treatment status.

***Data collection and M2BPGi measurement***

Data including demographics, comorbid diseases, laboratory tests, and other pertinent radiological, pathological and clinical information at baseline and follow-up were extracted from each clinical centers and were recorded using the same data frame and variable definitions. One investigator reviewed the datasets and audited the accuracy. Cirrhosis was determined via histology or by clinical criteria mainly composed of radiological features (nodular hepatic surface, coarse echotexture, irregular vasculature, and splenomegaly) 16.

Noninvasive scores based on routinely available laboratory tests were also used to estimate levels of liver fibrosis and hepatic dysfunction. The scores of the aspartate aminotransferase (AST) to platelet ratio index (APRI), Fibrosis 4 (FIB-4), and MELD (model for end-stage liver disease) were calculated according to the following equations: [AST/38 (U/L) /platelet count (103/μL)] × 100, [AST (U/L) × Age (years)]/[ALT (U/L)1/2 × platelet count (103/μL)], and 3.78×ln[serum bilirubin (mg/dL)] + 11.2×ln[INR] + 9.57×ln[serum creatinine (mg/dL)] + 6.43, respectively 17-19.

Serum M2BPGi levels were measured using sera collected prior to NA initiation, at 1 year and at 2 years after the therapy. Archived sera from the 2 hospitals were sent to a single laboratory (Nagoya City University, Nagoya, Japan) for the measurement of M2BPGi. The protocol has been reported previously 20. In brief, it was measured by an automated analyzer applying the lectin-antibody sandwich immunoassay (HISCL-2000i; Sysmex Corporation, Hyogo, Japan). The quantity of M2BPGi that was conjugated to *Wisteria floribund*a agglutinin was expressed in cut-off index (COI) and calculated by the formula: (M2BPGisample – M2BPGinegative control) / (M2BPGipositive control – M2BPGinegative control). For the purpose of standardized calibration, the supplied solution of positive control would yield a COI of 1.0.

***Longitudinal follow-up and outcome measure***

The primary study outcome was the development of incident HCC. The surveillance for HCC was carried out by abdominal sonography in principle, whereas computed tomography or magnetic resonance image served as the second-line modality for diagnostic confirmation 21. Generally, the frequency of surveillance was every 6 months in patients without cirrhosis and 3 months in those with cirrhosis. Patients were censored at loss to follow-up, death, or end of the observation in the end of September 2017. HCC was diagnosed via histology, cytology, or noninvasive criteria using dynamic images as recommended by the American Association for the Study of Liver Diseases 21.

***Statistical analysis***

Continuous variables were expressed as medians and interquartile ranges (IQR) and categoricalvariables as percentages. Serial M2BPGi levels were first explored by the Skillings–Mack test which accounted for missing data in analysis with repeated measurements 22. The differences between the respective serum M2BPGi levels were further examined by the Wilcoxon signed-rank test in patients with available sera at all three time points.

The cumulative incidence of HCC was estimated by the Kaplan Meier method. We performed the Cox proportional hazard regression to evaluate the association between M2BPGi levels and subsequent development of HCC. The measurements of M2BPGi at different time points were examined individually and compared against each other for the association with HCC. M2BPGi levels were also analyzed as a time-varying variable in the model.

Next, we developed a risk score based on the most predictive M2BPGi for HCC as well as other risk factors significantly associated with HCC in the multivariable Cox model. The model examined all potential predictors regardless of the results in the univariable analyses and was determined by stepwise elimination to remove insignificant factors. The predictive factors in the risk score were weighted according to their regression coefficients. We constructed time-dependent receiver operating characteristic (ROC) curves for censored survival data to appraise the risk score that included M2BPGi as an explanatory variable.

All data analysis was performed using either the Stata software (13.0 version, College Station, Texas) or the R program (version 3.4.0). All statistical tests were two-tailed and a *p* value less 0.05 defined statistical significance.

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