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Quantitation of anti-HBe antibodies in anti-HBc-positive liver donors

To the Editor:

There is increasing interest in quantitatively determining the presence of antibodies to hepatitis B virus (HBV) antigens both in patients with overt HBV infection and in those with occult HBV infection.[1,2] Recently, we studied a cohort of 100 hepatitis B surface antigen (HBsAg)-negative/anti-HBc-positive liver donors and found that levels of anti-HBc of IgG class above a 4.4 cut-off index (COI) correlated with the finding of intrahepatic HBV covalently closed circular (ccc) DNA, providing a novel potential tool to identify individuals with occult HBV infection (OBI) at increased risk of HBV reactivation following pharmacological immunosuppression.[3] Consistent with our results, Bae *et al.*[4] showed that among patients with resolved HBV infection undergoing allogenic hematopoietic stem cell transplantation, the finding of total anti-HBc with a titer ≥ 8 signal-to-cut-off (S/CO) ratio was independently predictive of HBV reactivation (hazard ratio [HR] 7.429, $p = 0.002$). Similarly, Yang *et al.*[5] observed that a high titer of total anti-HBc (≥6.41 IU/ml) and low anti-HBs (<56.48 mIU/ml) were significantly associated with HBV reactivation (HR 8.48 and 4.52, respectively, $p < 0.010$) in lymphoma patients with resolved HBV infection undergoing chemotherapy. However, while in the previous study, no difference was observed in anti-HBe positivity between patients with HBV reactivation and those without (39.3% *vs.* 41.7%, respectively, $p = 0.827$),[5] in our series the rate of anti-HBe positivity was significantly different between HBV cccDNA-positive and -negative individuals (55.6% *vs.* 23.3%, respectively, $p = 0.003$). On this premise, we further investigated the possible role of anti-HBe quantitation as a surrogate marker of intrahepatic HBV cccDNA in the same cohort of 100 anti-HBc-positive liver donors.[3]

The measurement of anti-HBe was performed by chemiluminescent enzyme immunoassay (CLEIA) on the fully automated system Lumipulse® G600 II (Fujirebio, Tokyo, Japan). The antibody quantitation was performed using an anti-HBe assay (Lumipulse® G HBeAb-N) calibrated against the WHO 1st International Standard for anti-HBe (Paul-Ehrlich-Institut, Langen, Germany) (Fig. 1A). Lower limit of detection (LLoD) and lower limit of quantitation (LLoQ) were estimated at 0.31 IU/ml and 0.35 IU/ml, respectively (Fig. 1B). The accuracy of recovery was 98.8% (95% CI 94.4%–103.3%) (Table S1). The coefficient of variation for repeatability of the assay (intra-run variation) was 3.1%, whereas for reproducibility (inter-run variation) it was 4.0%. Dilution parallelism of standard curve and anti-HBe-positive serum sample is reported in Fig. S1. Statistical analyses were performed using MedCalc® software, version 16.8. (MedCalc, Ostend, Belgium).

Among the 32 anti-HBe-positive donors, antibody values ranged from < 0.35 IU/ml to 14.8 IU/ml, with a median of 0.60 IU/ml. In the whole cohort of 100 liver donors, anti-HBe quantitation showed an area under the curve (AUC) of 0.713 (95% CI 0.614–0.799, $p < 0.001$) for the discrimination between HBV cccDNA-positive and -negative liver specimens; an anti-HBe titer > 0.68 IU/ml (sensitivity = 44.4% and specificity = 95.9%) allowed us to predict the presence of HBV cccDNA in the liver with a positive predictive value of 80.0%. For anti-HBc IgG, 4.4 COI was the optimal cut-off that maximized sensitivity (92.6%) and specificity (48.0%) (AUC = 0.680, 95% CI 0.577–0.771, $p = 0.002$), with a negative predictive value of 94.6%.[4] In a multivariate logistic regression model including age, gender, anti-HBe > 0.68 IU/ml and anti-HBc IgG > 4.4 COI, both antibodies were significantly and independently associated with measurable intrahepatic HBV cccDNA (odds ratio = 11.641, $p = 0.001$ and odds ratio = 7.239, $p = 0.016$, respectively) (Table 1).

We previously observed that the quantitation of anti-HBc IgG allowed the presence of intrahepatic HBV cccDNA to be ruled out in 37 out of 100 anti-HBc-positive liver donors (negative predictive value = 94.6%)[4] yet the serological prediction of

Keywords: Hepatitis B virus; HBV cccDNA; Occult HBV infection.

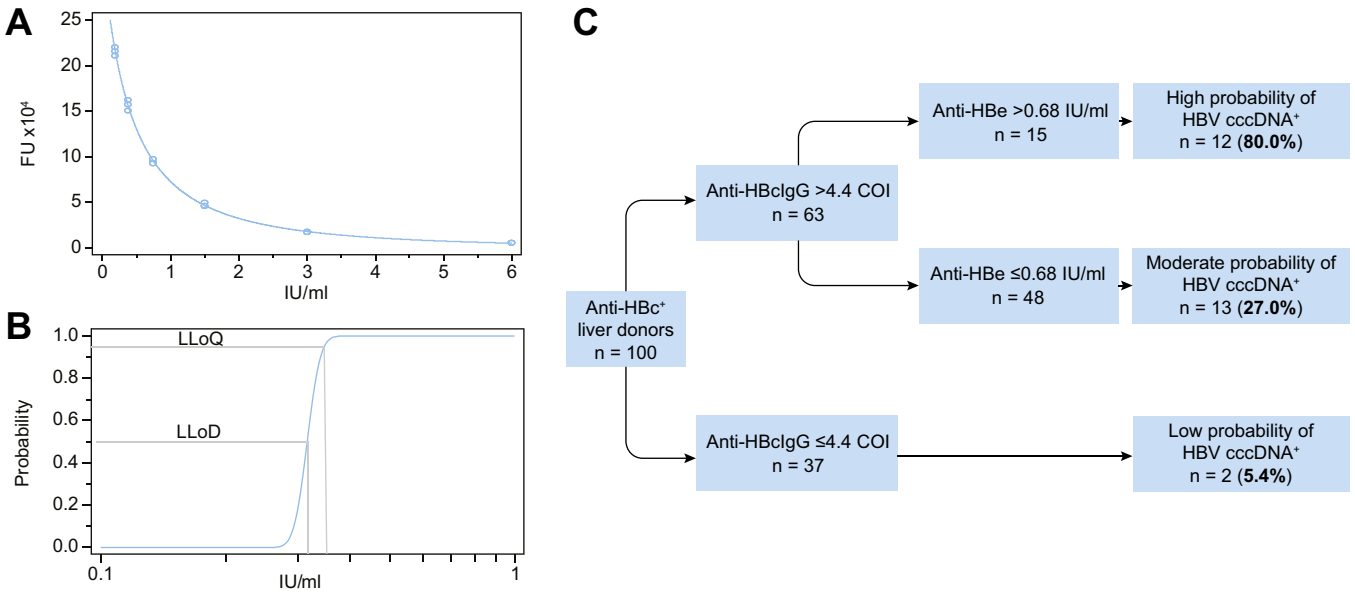

Fig. 1. Development and clinical application of the quantitative anti-HBe assay. (A) Anti-HBe standard curve defined by a non-linear regression model. (B) Probit analysis sigmoid curve reporting the LLoD and the LLoQ of the assay. (C) Diagnostic flow diagram for the prediction of HBV cccDNA presence in the liver of anti-HBc-positive individuals according to anti-HBc IgG and anti-HBe values. Anti-HBc, antibody to hepatitis B core antigen; anti-HBe, antibody to hepatitis B e antigen; cccDNA, covalently closed circular DNA; COI, cut-off index; FU, fluorescence units; HBV, hepatitis B virus; LLoD, lower limit of detection; LLoQ, lower

Table 1. Univariate and multivariate analyses of variables associated with the presence of intrahepatic HBV cccDNA in the 100 anti-HBc-positive liver donors.

Variables	HBV cccDNA– (n = 73)	HBV cccDNA+ (n = 27)	*p* value	OR (95% CI)	*p* value
Age, years*	70.8 (66.1–72.9)	63.0 (53.3–70.3)	0.049	0.957 (0.916–1.001)	0.054
Gender, M	49 (67.1%)	15 (55.6%)	0.350	0.859 (0.278–2.684)	0.794
Anti-HBs, mIU/ml*	56.0 (27.3–97.7)	58.0 (25.8–184.3)	0.481	–	–
Anti-HBc IgG, COI*	5.7 (3.6–9.7)	17.0 (7.0–39.2)	0.007	7.239 (1.458–35.932)	0.016
Anti-HBe, IU/ml*	0 (0–0)	0.36 (0–0.77)	<0.001	11.641 (2.590–52.321)	0.001

* Data are expressed as median (95% CI). Continuous variables were compared by Mann-Whitney test whereas categorical variables were analyzed by Fisher's exact test. Anti-HBc, antibody to hepatitis B core antigen; Anti-HBe, antibody to hepatitis B e antigen; Anti-HBs, antibody to hepatitis B surface antigen; cccDNA, covalently closed circular DNA; COI, cut-off index; HBV, hepatitis B virus; M, male; OR, odds ratio.

intrahepatic HBV cccDNA remained elusive in the other 63 liver donors. This study has shown that the quantitation of anti-HBe further increases the probability of identifying HBsAg-negative individuals carrying HBV cccDNA in the liver (positive predictive value = 80.0%) (Fig. 1C).

Although antibody reactivity also depends on host factors, we may suppose that higher titers of anti-HBe, as well as anti-HBc IgG, reflect the residual quantity of viral antigens secreted in the blood and thus HBV cccDNA quantity/productivity, even in HBsAg-negative phase of chronic HBV infection.

In conclusion, the present findings appear relevant in the HBsAg-negative/anti-HBc-positive individuals undergoing immunosuppressive therapy or chemotherapy;[6] the possibility to further stratify the risk of viral reactivation by means of combined antibody markers may be useful to tailor their management with HBV antivirals.

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Conflict of interest

The authors declare no conflicts of interest that pertain to this work.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

Study concept and design: Gian Paolo Caviglia, Antonina Smedile. Performing experiments: Gian Paolo Caviglia, Antonella Olivero. Analysis and interpretation of data: Gian Paolo Caviglia, Francesco Tandoi, Mario Rizzetto, Renato Romagnoli. Drafting the manuscript: Gian Paolo Caviglia, Francesco Tandoi. Critical revision of the manuscript for important intellectual content: Giorgio Maria Saracco, Mario Rizzetto, Renato Romagnoli, Antonina Smedile. Statistical analysis: Gian Paolo Caviglia.

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Supplementary data

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Diabetes mellitus as a risk factor of hepatocellular carcinoma in patients with chronic hepatitis B on nucleot(s)ide analogues

To the Editor:

We read with interest the article by Hsu and colleagues, who suggested that diabetes mellitus was a risk determinant for the prediction of hepatocellular carcinoma (HCC) in Asian patients with chronic hepatitis B, receiving antiviral therapy.[1] However, we have several concerns about the design and analysis of the study.

The time relationship and association between diabetes, chronic liver disease and development of HCC have not been corroborated in areas with high prevalence of chronic viral hepatitis. It is controversial whether diabetes is a risk factor for HCC in Taiwan. Most studies indicated that it was not a risk factor for HCC.[2–6] A possible link was reported in some subgroups.[7–9] However, using the same database,[1] another population-based study reported that diabetes increased HCC risk.[10]

This study used the National Health Insurance database in Taiwan.[1] It was not designed as a randomized clinical trial for research purpose. It was a retrospective and an observational study, thus selection bias was possible. The definition of disease was exclusively based on claim data and coding with the International Classification of Diseases, Ninth Revision, without laboratory data to confirm the diagnosis. The diagnostic accuracy is unknown. Nevertheless, errors in coding or recording tend to occur at random as a result of the same codes being used in both groups of patients (diabetes and non-diabetes). There might be many biases in both the risk and the outcome of interest (diabetes and HCC), such that statistical association could not be well verified. Though most patients with a diagnosis of diabetes were likely to have actual diabetes, those without a diabetes diagnosis might have had diabetes but not been recognized. Additionally, cirrhosis itself could have driven the development of diabetes, which might have been clinically silent and therefore gone undetected. Meanwhile, cryptogenic cirrhosis derived from diabetes might be a misclassified bias which might modify the true effects of diabetes on the risk of HCC. Additionally, it was hard to confirm whether diabetes had a causative role in HCC or whether both diseases were the product of other factors, particularly chronic liver disease.

Several issues remain unresolved. Other possible confounding factors, such as alcohol drinking and smoking, diabetes-induced metabolic changes (dyslipidemia, steatohepatitis and fibrosis) and antidiabetic medications have not well been analyzed.

Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

Please refer to the accompanying ICMJE disclosure forms for further details.

Supplementary data

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