

Letter to the Editor

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Diagnostic and prognostic value of quantitative detection of antimitochondrial antibodies subtype M2 using chemiluminescence immunoassay in primary biliary cholangitis

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To the Editor,

Primary biliary cholangitis (PBC) is an autoimmune liver disease characterized by cholestasis, disease-specific autoantibodies, and histologic evidence of chronic non-suppurative destructive cholangitis affecting small or medium-sized bile ducts [1]. Without appropriate treatment, the disease can be progressive and lead to liver cirrhosis and liver failure [2]. Antimitochondrial antibody (AMA), the most specific autoantibody, is used as a serological marker for the diagnosis of PBC. The most specific subtype for PBC is the M2 subtype of AMA (AMA-M2) [2]. Indirect immunofluorescence (IIF) is the most common method for detecting AMA, using either rat triple-tissue sections (liver-kidney-stomach) or the HEp-2 cell line as substrates. Clinically, various methods are used to detect AMA-M2, such as enzyme-linked immunosorbent assay (ELISA), fluorescent bead-based assays, and line immunoassay (LIA). A previous meta-analysis found that both IIF-AMA and AMA-M2 have favorable accuracy for diagnosing PBC [3]. Moreover, combining the detection of IIF-AMA and AMA-M2 can achieve the highest sensitivity [4]. Chemiluminescence immunoassay has gained increasing attention in clinical

diagnosis due to its high sensitivity, good specificity, wide linear range, and other desirable characteristics. However, research on the detection of AMA-M2 using chemiluminescence immunoassay is scarce. Therefore, we conducted a study to assess the diagnostic and prognostic value of quantitative detection of AMA-M2 using chemiluminescence immunoassay in PBC.

This study involved 158 PBC patients and 20 negative controls, including 5 cases each of alcoholic liver disease, hepatitis B, hepatitis C, and drug-induced liver injury. PBC was diagnosed according to the American Association for the Study of Liver Diseases (AASLD) guidelines [5]. AMA was detected using the Euroimmun test kit (EUROIMMUN, Germany) with rat kidneys as a substrate through IIF. PBC-related autoantibodies, such as AMA-M2, M2-3E, anti-Sp100, and anti-gp210 antibodies, were detected using the Euroimmune Test System (EUROIMMUN, Germany) through LIA. The discarded biological samples of these 178 patients were preserved and quantitatively detected for AMA-M2. Prior to reuse, the participants signed a consent form indicating their agreement to the reuse of their specimens for research purposes. This study was approved by the Ethics Committee of Hangzhou Xixi Hospital (2023048).

The iFlash-AMA-M2 assay (YHLO, China) and iFlash 3000 Chemiluminescence Immunoassay Analyzer (YHLO, China) were used to quantitatively determine AMA-M2 through chemiluminescent immunoassay. The iFlash-AMA-M2 assay is an indirect immunoassay that measures the chemiluminescent reaction as relative light units (RLUs). The amount of AMA-M2 in the sample is directly proportional to the RLUs detected by the iFlash optical system. Results are determined by using an instrument-specific calibration curve generated through a 3-point calibration and a master curve provided via the reagent QR code.

The area under the ROC curve for diagnosing PBC using iFlash-AMA-M2 was 0.842 (95 % CI 0.776–0.907). When a cut-off value of 13.5 AU/mL, corresponding to the highest Jordan Index, was used, the sensitivity and specificity were

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66.5 and 95.0 %, respectively. According to the manual, nonreactive is defined as <16 AU/mL, indeterminate as ≥ 16 to <24 AU/mL, and reactive as ≥ 24 AU/mL. When a cut-off value of 16 AU/mL was used, the sensitivity and specificity were 63.9 and 95.0 %, respectively. Using a cut-off value of 20 AU/mL resulted in a sensitivity and specificity of 57.6 and 100 %, respectively. Lastly, when a cut-off value of 24 AU/mL was used, the sensitivity and specificity were 51.9 and 100 %, respectively.

The agreement between methods was assessed using the Kappa coefficient, with values ranging from 0 to 1. A Kappa value of 0–0.2 represents slight agreement, 0.2–0.4 represents fair agreement, 0.4–0.6 represents moderate agreement, 0.6–0.8 represents substantial agreement, and 0.8–0.9 represents excellent agreement. Table 1 shows agreement between iFlash-AMA-M2 and other AMA detection methods using various iFlash-AMA-M2 cutoff values (16, 20, and 24 AU/mL). At different cutoff values, iFlash-AMA-M2 and other AMA detection methods, including IIF and LIA, exhibited almost excellent agreement. At a cutoff value of 16 AU/mL, iFlash-AMA-M2 and M2-3E (LIA) showed the highest consistency with a value of 93.8 % and a Kappa coefficient of 0.874. Moreover, at a cutoff value of 24 AU/mL, iFlash-AMA-M2 had the highest agreement with AMA-M2 (LIA) at 88.2 % (kappa value of 0.756). One possible explanation for this phenomenon is that the chemiluminescence assay is more sensitive in detecting AMA-M2 than LIA. As shown in Supplementary Material, Table S1, increasing the cutoff value of iFlash-AMA-M2 converts false negative results of AMA-M2 (LIA) to true negative results, improving consistency between the two methods. In addition, even among the 76 patients classified as iFlash-AMA-M2 negative (<16 AU/mL) according to the manual, there were still 10 cases positive for IIF-AMA and 4 cases positive for M2-3E (LIA), with 3 cases positive for both. Supplemental Material, Table S2 demonstrates that combining multiple AMA detection methods can improve both the sensitivity and accuracy of AMA detection. Thus, in patients with suspected PBC, it may be necessary to combine multiple methods to improve the detection sensitivity of AMA.

Of 158 PBC patients, there were 94 cases of liver cirrhosis and 64 cases without liver cirrhosis. Liver cirrhosis was diagnosed according to the Chinese guidelines on the management of liver cirrhosis [6]. Figure 1 shows the correlation between iFlash-AMA-M2 and logit-transformed cirrhosis incidence. The results indicated that iFlash-AMA-M2 was almost linear. Table 2 demonstrates that iFlash-AMA-M2 was significantly associated with the occurrence of liver cirrhosis as a continuous variable, as determined by performing logistic regression (OR: 1.008, 95 % CI: 1.004–1.013). Moreover,

Table 1: Agreement between iFlash-AMA-M2 and other AMA detection methods using various iFlash-AMA-M2 cutoff values.

iFlash-AMA-M2 cutoff value, AU/mL	Methods	Agreement, %	Kappa value
16	IIF-AMA	87.1	0.737
20		87.6	0.752
24		83.7	0.678
16	AMA-M2 (LIA)	75.8	0.540
20		82.0	0.643
24		88.2	0.756
16	M2-3E (LIA)	93.8	0.874
20		93.3	0.865
24		87.1	0.745

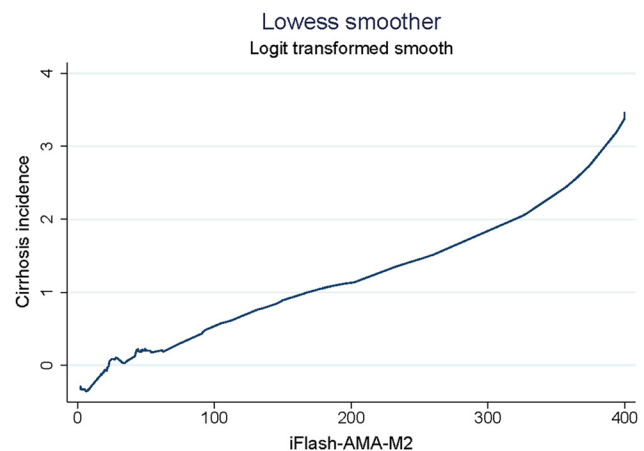


Figure 1: Relationship between iFlash-AMA-M2 and logit transformed cirrhosis incidence by using lowess smoothing technique.

Table 2: Logistic regression analysis of the association between iFlash-AMA-M2 and cirrhosis.

	Odds ratio	95 % CI	
iFlash-AMA-M2, AU/mL	1.008	1.004–1.013	<0.001
iFlash-AMA-M2, AU/mL			
<20	Reference		
20–50	1.475	0.648–3.354	0.354
50–200	4.179	1.471–11.866	0.007
>200	20.893	4.609–94.713	<0.001
P for trend			<0.001

OR, odds ratio; CI, confidence interval.

categorizing iFlash-AMA-M2 into a design variable (<20 ; 20–50; 50–200; >200 AU/mL), with <20 AU/mL as the reference group, an increasing trend of OR values was observed with an increase of iFlash-AMA-M2.

In conclusion, our research found that the quantitative detection of AMA-M2 by chemiluminescence immunoassay has good diagnostic efficacy in the diagnosis of PBC, and good consistency with the detection results of IIF and LIA. Additionally, we found a significant correlation between AMA-M2 and the occurrence of liver cirrhosis in PBC patients.

Research ethics: This study was approved by the Ethics Committee of Hangzhou Xixi Hospital (2023048).

Informed consent: Informed consent was obtained from all individuals included in this study, or their legal guardians or wards.

Author contributions: The authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: The authors state no conflict of interest.

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Data availability: The raw data can be obtained on request from the corresponding author.

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