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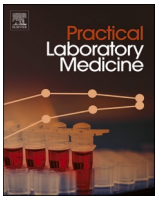

A comparison of chemiluminescent immunoassay and enzyme-linked immunosorbent assay for detecting phospholipase A2 receptor antibody in primary membranous nephropathy

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ABSTRACT

Objective: The accurate detection of phospholipase A2 receptor (PLA2R) autoantibody is crucial in the diagnosis and monitoring of primary membranous nephropathy (pMN). While enzyme-linked immunosorbent assay (ELISA) is the commonly used detection method, its complexity and time-consuming nature pose challenges, especially for small sample sizes. Chemiluminescence immunoassay (CLIA) has emerged as a rapid alternative for clinical immunoassays. This study aims to compare the sensitivity, specificity, and precision of CLIA and ELISA in detecting PLA2R autoantibody.

Method: A total of 145 patients with biopsy-confirmed primary membranous nephropathy and 85 patients with non-membranous nephropathy were enrolled in this comparative study. CLIA and ELISA were employed to test all samples for the presence of PLA2R autoantibodies. Statistical analysis of sensitivity, specificity, accuracy, positive predictive value (PPV), and negative predictive value (NPV) was performed using SPSS 26.0. The diagnostic value of ELISA and CLIA for pMN was analyzed using the ROC curve, and Correlation analysis was performed using Spearman.

Results: Serum levels of anti-PLA2R antibody in pMN group were significantly higher than those in nMN group($P < 0.05$). The accuracy of CLIA for detecting anti-PLA2R antibody was 76.96%, while ELISA showed an accuracy of 74.78%. The sensitivity for CLIA was 64.83%, compared to 60% for ELISA. However, no statistically significant difference was observed between the two methods ($P > 0.05$). The overall qualitative agreement of anti-PLA2R detection was 93.35% (95% confidence interval[CI] 89.47–96.3). ROC curve analysis showed that AUC of anti-PLA2R antibody detected by ELISA and CLIA were 0.8737 (95% confidence interval [CI] 0.8270–0.9204), 0.8914 (95% confidence interval [CI]0.8495–0.9332), respectively. The Spearman correlation analysis revealed a significant correlation between them($P < 0.05$). Notably, CLIA demonstrated a significant time-saving advantage, particularly when the sample size was less than 200, and especially when it was less than 20.

Conclusion: CLIA and ELISA showed similar accuracy and consistency in detecting anti-PLA2R antibody for primary membranous nephropathy. However, CLIA exhibited a significant

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advantage in terms of automation and time-saving compared to ELISA, particularly for smaller sample sizes. This finding suggests that CLIA has the potential to become a preferred and widely adopted test in the future.

1. Introduction

Membranous nephropathy (MN) is the leading cause and pathological subtype of nephrotic syndrome in adults. It is characterized by a gradual progression and a wide range of clinical presentations. Studies have shown that approximately one-third of MN patients will ultimately develop end-stage kidney disease (ESKD), underscoring the significance of early detection and intervention [1]. Traditionally, the diagnosis of MN has relied on renal biopsy, which is an invasive and intricate procedure. However, challenges arise when patients have contraindications to biopsy, complicating the diagnostic process. Since 2009, an increasing body of evidence has established a strong association between the presence of M-type phospholipase A2 receptor autoantibody (PLA2R) and MN, particularly primary membranous nephropathy (pMN) [2]. Studies have shown that the majority of pMN cases, ranging from approximately 70%–80%, are positive for PLA2R antibody [3]. The 2021 clinical practice guideline by the Kidney Disease: Improving Global Outcomes (KDIGO) for managing glomerular diseases recommends utilizing anti-PLA2R antibody as a serological marker for diagnosing pMN [4]. In instances where MN patients exhibit a positive PLA2R antibody test, have normal kidney function, and negative findings in secondary MN screening, renal biopsy may be deemed unnecessary [4]. Furthermore, monitoring anti-PLA2R antibody levels can serve as a valuable tool in assessing disease activity, evaluating treatment response, and providing prognostic insights.

Enzyme-linked immunosorbent assay (ELISA) is a highly sensitive test technique which combines the specific reaction of antigen and antibody with the efficient catalysis of enzyme. ELISA has emerged as the current preferred clinical laboratory test due to its ability to accurately quantify the concentration of anti-PLA2R antibody [5]. However, it has limitations such as long detection times and high labor costs, which can hinder its practical application. In recent years, novel detection techniques have been increasingly utilized in clinical autoantibody detection experiments. One such method is Chemiluminescence Immunoassay (CLIA), which combines chemiluminescence and immunoassay principles [6]. CLIA comprises the following steps: a. labeling an antigen or an antibody with a chemiluminescence-related substance; b. separating a free chemiluminescence-related marker after a specific antigen-antibody reaction; c. adding other related substances of a chemiluminescence-related system to generate chemiluminescence; d. carrying out qualitative or quantitative detection on the labeled antigen or antibody. CLIA has gained widespread use in clinical disease diagnosis, particularly for tumor biomarker and autoantibody detection, owing to its rapid detection speed, ease of operation, and high sensitivity and specificity [7,8]. As a result, it could be applied in pharmaceutical control, clinical diagnostics, and environmental monitoring. It is the best alternative to enzyme-linked immunoassay and radioimmunoassay. This study aimed to compare the diagnostic performance of CLIA and ELISA in detecting serum anti-PLA2R antibody, with the objective of identifying a more accurate, rapid, automated, and convenient method for clinical use.

2. Materials and methods

2.1. Patients and samples

A total of 230 patients who underwent renal biopsy in the Second Affiliated Hospital of Xi'an Jiaotong University from January 2020 to July 2022 were included in this study. Among them, 145 patients were diagnosed with primary membranous nephropathy (pMN) while 85 patients had non-membranous nephropathy (nMN). pMN is diagnosed when polyclonal immunoglobulin G (IgG; often IgG4 dominant), granularly deposits capillary wall with variable C3, and corresponding positive granular capillary wall PLA2R staining, a "spike" appearance is visualized by light microscopy,and extensive foot process effacement and subepithelial deposits by Electron microscopy [9]. In the pMN group, there were 91 male patients (62.76%) and 54 female patients (37.24%), with ages ranging from 17 to 75 years [median age: 52 (37–59.5)]. The nMN group consisted of patients with various conditions, including IgA nephropathy (n = 35), mesangial proliferative glomerulonephritis (n = 18), focal segmental glomerulosclerosis (n = 8), nodular diabetic glomerulosclerosis (n = 6), lupus nephritis (class III n = 2, class IV n = 3, class V n = 1), minimal change disease (n = 5), atypical membranous nephropathy (n = 2), hypertensive renal impairment (n = 2), membranous proliferative glomerulonephritis (n = 1), focal proliferative necrotic glomerulonephritis (n = 1), and capillary proliferative glomerulonephritis (n = 1). This group consisted of 42 male patients (49.41%) and 43 female patients (50.59%), with ages ranging from 15 to 76 years [median age: 44 (32.25–54.75)].

Venous blood samples of at least 3 ml were collected from all patients prior to renal biopsy. The samples were centrifuged at 3000 r/min for 5 min within 2 h to get 500 μl serum. The serum samples were then frozen at −80 °C until the PLA2R testing was performed. The concentration of anti-PLA2R antibody was measured using both CLIA and ELISA methods (specific procedures described below). It is important to note that the blood samples were not subjected to repeated freezing and thawing. Clinical data were retrospectively collected from the hospital information system. The study protocol was approved by the Ethics Review Committee of the Second Affiliated Hospital of Xi'an Jiaotong University (ethics approval number: 2022-780).

2.2. Immunassays

2.2.1. ELISA detection of anti-PLA2R antibodies

The concentration of anti-PLA2R antibody was measured using the anti-PLA2R ELISA (IgG) Kit from Omnimedical Diagnostics AG, Germany. The ELISA assay was conducted following the instructions provided by the manufacturer. An automated spectrophotometer was used to read the optical density of the samples at a wavelength of 450 nm (RT-6100, Shenzhen Leidu Life Sciences Co., Ltd, China), as shown in Fig. 1A. In accordance with the manufacturer's recommendations, results were interpreted as negative if the value was less than 20 relative units (RU)/ml and positive if it was ≥20 RU/ml.

2.2.2. CLIA detection of anti-PLA2R antibody

The serum anti-PLA2R antibody levels were assessed using the anti-PLA2R antibody IgG kit (Sichuan Crip Light Biotechnology Co., Ltd, China) on a fully automated chemiluminescence analyzer. The testing procedure strictly followed the manufacturer's instructions, as shown in Fig. 1B. We applied a cut-off value of 20 RU/ml to determine the presence or absence of anti-PLA2R antibody according to manufacturer protocol. It's important to note that both the CLIA and ELISA tests were performed by the same personnel, ensuring consistency and adherence to the instructions provided by the manufacturers.

2.3. Statistical analysis

The statistical analysis of the data was performed using SPSS 26.0 statistical software (IBM SPSS, Chicago, IL, USA). The categorical variables were described as percentages and then analyzed using the $\chi 2$ test or Fisher's precision probabilitytest. The normally distributed data were presented as means ± standard deviation (SD) and were compared using independent t-tests between the pMN and nMN groups. Non-normally distributed data were expressed as median (25th-75th interquartile range), and the Mann-Whitney test or Kruskal-Wallis test was performed for statistical comparison. Cohen's kappa test was used to analyze the agreement between portions, with kappa (k) values corresponding to different levels of agreement. A P value of less than 0.05 was considered statistically significant. Specifically, kappa values of 0.81–1.00 indicated almost perfect agreement, 0.61–0.80 indicated substantial agreement, 0.41–0.60 indicated moderate agreement, 0.21–0.40 indicated fair agreement, 0.01–0.20 indicated slight agreement, and ≤0 indicated no agreement. Spearman correlation analysis was employed to identify the correlation between the methods. The receiver operating characteristic curve (ROC curve) and images were designed by GraphPad Prism 8.0.

3. Results

3.1. Clinical baseline characteristics

A total of 230 patients were included in the study, with 145 patients in the pMN group and 85 patients in the nMN group. The composition of hypertension and diabetes mellitus did not show any significant differences between the two groups ($P > 0.05$), as shown in Table 1. The male-to-female ratio in the pMN group was 1.69:1, which is consistent with findings from previous studies [10, 11]. In terms of age, the median age in the pMN group was 52 years, which was higher than the median age of 44 years in the nMN

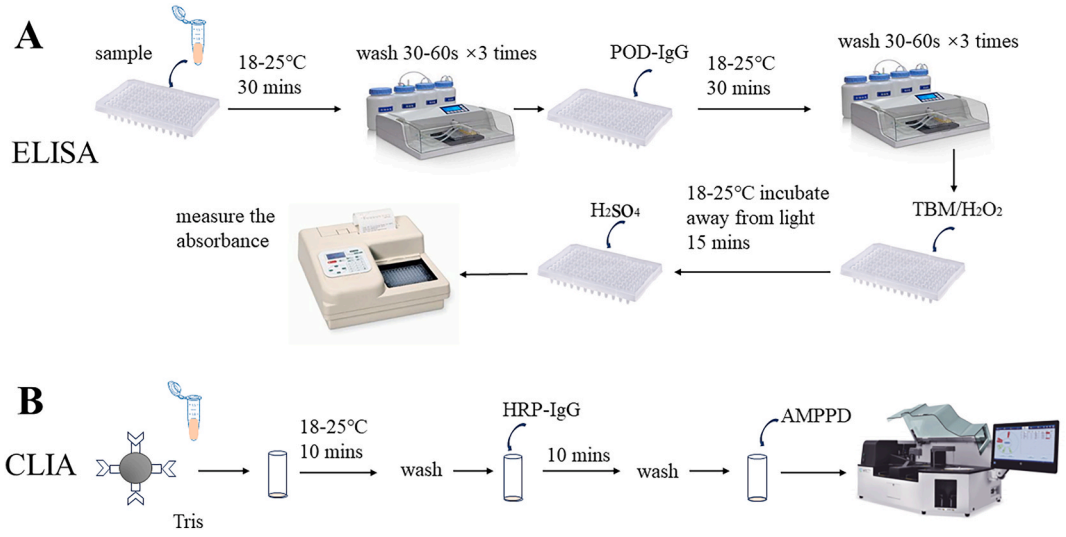

Fig. 1. Procedure for ELISA and CLIA
POD:peroxidase, TBM/H2O2: 3,3′,5,5′-tetramethylbenzidine/hydrogen peroxide, HRP: horseradish peroxidase, AMPPD: 3-[2-spiroadamatane]-4-methoxy-4-[3-phosphoryloxy]-phenyl-1,2-dioxetane) Dioxetane

Table 1
Demographic and biochemical baseline characteristics.

	pMN	nMN	Statistical quantities	*P*-value
No.of Patients	145	85	–	–
Age (y)[b]	52(37–59.5)	44(32.25–54.75)	−2.82	<0.05
Sex (M/F)	91/54	42/43	3.91	<0.05
Hypertension [n (%)]	43(29.66%)	32(37.65%)	1.56	>0.05
Diabetes mellitus [n (%)]	15(10.34%)	12(14.12%)	0.74	>0.05
UTP (mg/24h)[b]	5462.8(3045.71–8249.2)	2566.32(1304.28–5364.3)	−5.51	<0.05
BUN (μmol/L)[b]	4.7(3.85–6.05)	5.2(3.95–7.35)	−2.13	<0.05
BUA (μmol/L)[a]	354.21 ± 99.84	384.71 ± 119.81	−2.07	<0.05
eGFR(ml/min/1.73m^2)[b]	109.8(97.45–125.26)	107.7(66.32–124.79)	−1.61	>0.05
Scr (μmol/L)[b]	58.85(47.85–72.46)	63.07(48.61–102.49)	−1.92	>0.05
Triglycerides(mmol/L)[b]	2.15(1.54–3.16)	1.63(1.31–2.28)	−3.45	<0.05
Cholesterol (mmol/L)[b]	7.13(6.13–8.81)	4.96(3.9–6.91)	−6.25	<0.05
TP (g/L)[b]	49.5(41.45–54.65)	57(48.75–67.1)	−5.07	<0.05
Alb (g/L)[b]	25.2(21.55–29.4)	31.4(26.43–38.88)	−5.56	<0.05

M male, F female, UTP 24-h urine protein, BUN blood urea nitrogen, BUA blood urea acid, eGFR estimated glomerular filtration rate (CKD-EPI), Scr serum creatinine, TP total protein, Alb albumin.
[a] Mean ± SD.
[b] Median (25–75% interquartile range).

group, and this difference was statistically significant. This observation aligns with the fact that membranous nephropathy is more commonly seen in middle-aged and elderly men. Significant differences were found between the pMN and nMN groups in terms of 24-h urine protein, plasma albumin, total protein, triglycerides, and total cholesterol, with higher values observed in the pMN group. This further supports the understanding that MN is the most prevalent pathological subtype of nephrotic syndrome in adults [12].There is no significant difference in uric acid and creatinine between the two groups ($P > 0.05$). There were no significant differences in uric acid and creatinine levels between the two groups ($P > 0.05$).

3.2. Comparison of the diagnostic efficacy of the two methods

The median anti-PLA2R antibody in pMN patients measured by ELISA was 49.39 RU/ml (interquartile range [IQR], 3.11–187.90 RU/ml), significantly higher than other nephropathy patients (range 0.88–6.63 RU/ml; median concentration 2.02 RU/ml [IQR 1.78–2.29 RU/ml]). The difference was statistically significant ($P < 0.0001$). Antibody in pMN patients measured by CLIA varied between 2.00 RU/ml and 1500 RU/ml, with a median antibody level of 74.46 RU/ml (interquartile range [IQR], 3.48–222.20 RU/ml), significantly different from the control group (range 2.00–34.63 RU/ml; median concentration 2.00 RU/ml; interquartile range [IQR], 2.00–2.00 RU/ml, $P < 0.0001$) (Fig. 2).

In the pMN group, 87 cases were positive for anti-PLA2R antibody using ELISA, while 94 cases were positive using CLIA. Interestingly, CLIA also detected 2 cases of PLA2R antibody positivity in nMN patients who were negative by ELISA (Table 2 & Table 3).

The sensitivity of ELISA for detecting anti-PLA2R antibody in pMN patients was 60%, with a specificity of 100%. The accuracy, positive predictive value, and negative predictive value of ELISA were 74.78%, 100%, and 59.44%, respectively. On the other hand, CLIA showed a sensitivity of 64.83% and a specificity of 97.65%. The accuracy, positive predictive value, and negative predictive value of CLIA were 76.96%, 97.92%, and 61.94%, respectively. There were no statistically significant differences between the two methods in terms of their diagnostic performance ($P > 0.05$) (Table 3).

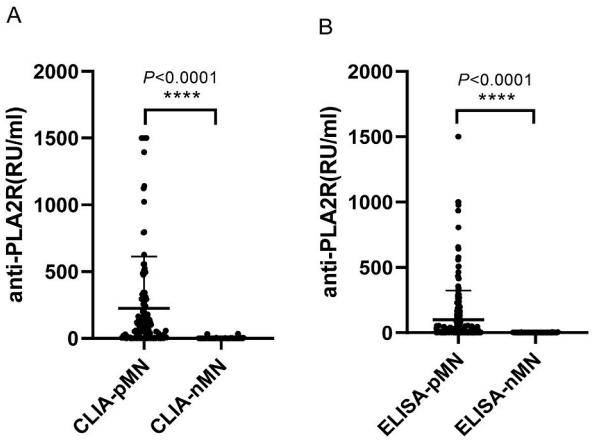

Fig. 2. Distribution of anti-PLA2R levels in pMN patients and controls by CLIA and ELISA.

Table 2
The results of ELISA and CLIA assay(n).

Testing Method		Pathology		Total
		pMN	nMN	
ELISA	Positive	87	0	87
	Negative	58	85	143
CLIA	Positive	94	2	96
	Negative	51	83	134
Total		145	85	230

Table 3
Diagnostic performance characteristics of ELISA and CLIA.

Testing Methods	pMN (n = 145)	nMN (n = 85)	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	Accuracy (%) (95% CI)	Positive predictive value (%) (95% CI)	Negative predictive value (%) (95% CI)
ELISA	87	85	60(51.54–68.04)	100 (95.75–100.00)	74.78 (68.65–80.26)	100(95.85–100.00)	59.44(50.92–67.56)
CLIA	94	83	64.83 (56.47–72.57)	97.65 (91.76–99.71)	76.96 (70.97–82.24)	97.92(92.68–99.75)	61.94(53.16–70.18)
$\chi 2$	–	–	0.72	0.506	0.297	–	0.181
P-value	–	–	0.396	0.477	0.586	[a]0.498	0.67

[a] Indicates that Fisher's exact probability method was used.

3.3. The consistency and correlation analysis between ELISA and CLIA

Furthermore, the consistency between CLIA and ELISA was assessed. The results demonstrated a positive coincidence rate of 96.55%, a negative coincidence rate of 91.61%, and an overall coincidence rate of 93.35% (Table 4). The calculated kappa (k) value was 0.864, indicating a good level of consistency between CLIA and ELISA in detecting anti-PLA2R antibody. The Spearman correlation analysis indicated significant correlation between CLIA and ELISA ($r = 0.674$, $P < 0.0001$; Fig. 3).

3.4. Evaluating diagnostic performance with ROC curves

To definite the value of anti-PLA2R antibody detected by ELISA and CLIA, we performed ROC curve analysis. Antibodies tested by ELISA (AUC = 0.8737 [95%CI, 0.8270–0.9204]; $P < 0.0001$) and CLIA (AUC = 0.8914 [95%CI, 0.8495–0.9332]; $P < 0.0001$) were statistically significant in the differential diagnosis of pMN and nMN. However, the difference in AUC was not significant ($P > 0.05$). Compared with 24-h urine protein (AUC = 0.7172 [95%CI, 0.6489–0.7855], $P < 0.0001$), albumin (AUC = 0.7249 [95%CI, 0.6541–0.7956]; $P < 0.0001$), total cholesterol (AUC = 0.7471 [95%CI, 0.6773–0.8169]; $P < 0.0001$), triglycerides (AUC = 0.6361 [95%CI, 0.5617–0.7106]; $P < 0.001$), eGFR (AUC = 0.5637 [95%CI, 0.4801–0.6473]; $P > 0.05$), serum creatinine (AUC = 0.5757 [95%CI, 0.4950–0.6564]; $P > 0.05$) and blood uric acid (AUC = 0.5670 [95%CI, 0.4884–0.6455]; $P > 0.05$), the anti-PLA2R antibody measured by CLIA and ELISA showed greater AUC values, suggesting a better performance in differentiating pMN from nMN (Fig. 4).

3.5. Performance characteristics of CLIA and ELISA

Additionally, we conducted a comparison of the methodological characteristics between CLIA and ELISA. The findings revealed that CLIA exhibited highly automated features, resulting in significant time-saving benefits and ease of execution when compared to ELISA. Particularly noteworthy is that CLIA allows for measurements to be conducted at any time, making it particularly advantageous for sample sizes smaller than 20 (Table 5).

4. Discussion

pMN is a prevalent form of nephrotic syndrome in adults and is associated with the risk of chronic renal failure and thromboembolic events. This condition not only causes significant physiological and psychological harm to patients but also imposes a substantial

Table 4
Consistency analysis of ELISA and CLIA for detection of anti-PLA2R antibody.

CLIA (n)	ELISA (n)		Positive coincidence rate (%) (95% CI)	Negative coincidence rate (%) (95% CI)	Total coincidence rate (%) (95% CI)	χ^2 test		Consistency check	
	Positive	Negative				χ^2	*P*	κ	*P*
Positive	84	12	96.55(90.25–99.28)	91.61(85.80–95.59)	93.35(89.47–96.30)	172.88	<0.05	0.864	<0.05
Negative	3	131							

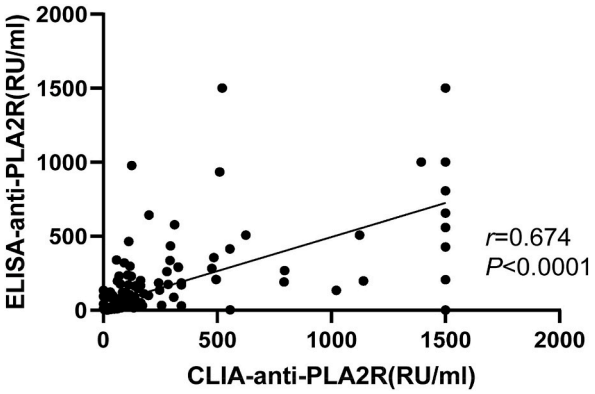

Fig. 3. Correlation between levels of anti-PLA2R antibody measured by ELISA and CLIA.
Correlation coefficients and *P* values were estimated using the Spearman's rank correlation test. Anti-PLA2R anti–phospholipase A2 receptor; CLIA chemiluminescence immunoassay; ELISA enzyme-linked immunosorbent assay; RU/ml relative units per milliliter.

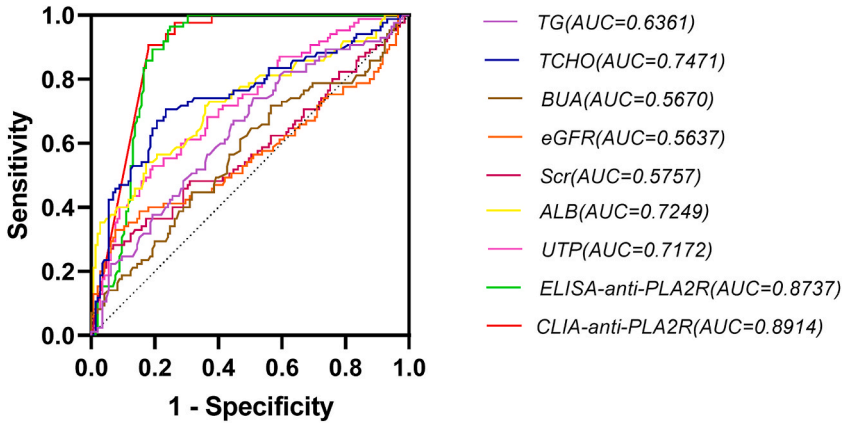

Fig. 4. ROC curve analysis
UTP 24-h urine protein; ALB albumin; Scr serum creatinine; eGFR estimated glomerular filtration rate (CKD-EPI); BUA blood urea acid; TCHO total cholesterol; TG triglycerides; Anti-PLA2R anti–phospholipase A2 receptor; CLIA chemiluminescence immunoassay; ELISA enzyme-linked immunosorbent assay.

Table 5
Comparison of the performance characteristics of ELISA and CLIA assays.

	ELISA	CLIA
Principle	Specific binding reaction of the antigen and antibody	A linear quantitative relationship between the concentration of the substance to be measured and the chemiluminescence intensity of the system
Steps (as show in Fig. 1)	Dilution-adding sample-incubation-washing-adding enzyme reactant-incubation- washing-adding substrate solution-incubation–adding termination solution- measurement-readings	Power on-adding sample- measurement-readings
Time consumption		
Measure 20 samples	120min	40min
Measure 50 samples	150min	100min
Measure 100 samples	250min	200min
Automation	Semi-automatic	Fully automatic

Time consumption refers to the time from sample addition to reading results.

economic burden on society [13]. The development of pMN is attributed to the presence of circulating autoantibodies targeting antigens on glomerular podocytes. As a result, immune complexes form and deposit on the glomerular basement membrane, leading to the activation of the complement system and disruption of the glomerular filtration barrier, ultimately resulting in proteinuria [10]. Currently, renal biopsy is the gold standard for diagnosing pMN. However, this invasive procedure carries potential complications, including perirenal hematoma, arteriovenous fistula, and infection. Additionally, certain conditions and contraindications, such as having a solitary kidney, abnormal coagulation function, poorly controlled hypertension, or uncooperative patients, limit the feasibility of renal biopsy [14]. As our understanding of the mechanisms underlying pMN continues to advance, non-invasive diagnostic approaches have emerged as valuable alternatives, revolutionizing the detection and management of pMN. These non-invasive methods play an increasingly important role in the early and accurate detection of the condition. By leveraging techniques such as ELISA or CLIA, healthcare providers can analyze blood samples to measure specific autoantibodies like anti-PLA2R antibodies. These approaches enable timely interventions and improve patient outcomes while minimizing the risks and discomfort associated with invasive procedures.

In 2009, Beck and colleagues made a significant discovery, confirming that PLA2R is the primary target antigen in pMN. They found that PLA2R is highly expressed in podocytes and co-expressed with IgG4 [2]. Subsequently, anti-PLA2R antibodies were detected in the serum of pMN patients for the first time using Western blot analysis [9]. Since then, numerous studies have demonstrated a close association between anti-PLA2R antibodies and the activity and progression of pMN. These antibodies have proven valuable in monitoring the response to immunosuppressive therapy and predicting the risk of recurrence in transplanted kidneys [15,16]. The 2021 KIDGO guidelines explicitly highlight the importance of anti-PLA2R antibodies in the diagnosis of MN, evaluation of disease activity, monitoring treatment effectiveness, and predicting the risk of recurrence after transplantation. According to the guidelines, patients with positive anti-PLA2R antibodies and normal renal function may not require renal biopsy. Anti-PLA2R antibody titers can be used to stratify the risk of MN, and longitudinal monitoring of anti-PLA2R antibody levels, particularly six months after initiating MN treatment, can provide valuable insights into treatment response [4]. In recent years, additional MN-associated antigens have been identified, with thrombospondin type-1 domain-containing 7A (THSD7A) being the most extensively studied [17]. Antibodies to THSD7A can be detected in approximately 3% of pMN patients. However, it is important to note that THSD7A cannot serve as a serological diagnostic marker for pMN.

In the detection of anti-PLA2R antibodies, various methods have been employed, including Western blot, indirect immunofluorescence (IIF), and ELISA. Western blot analysis is known for its high cost and complexity, while IIF results can be influenced by subjective factors. As a result, ELISA has emerged as the primary detection method due to its quantitative capabilities and straightforward procedure. However, ELISA still possesses certain limitations inherent to traditional immunological detection techniques, such as the need for multiple operational steps, extended time requirements, and high costs. Additionally, achieving rapid sample detection in clinical settings can be challenging with ELISA. To address these limitations, the CLIA detection technology has gained popularity as a new and mainstream clinical immunoassay method [7,8]. CLIA offers several advantages, including full automation, quantitative detection, and rapid results. It has found extensive application in the detection of disease-related markers in clinical settings, as well as in environmental and food testing [7]. Notably, there is a lack of comparative studies between ELISA and CLIA for the detection of anti-PLA2R antibodies. Consequently, this study aimed to compare CLIA and ELISA to assess their respective performance in detecting anti-PLA2R antibody.

The study findings revealed that ELISA exhibited a sensitivity of 60% and a specificity of 100% in detecting anti-PLA2R antibody, which is consistent with previous research conducted domestically and internationally [11,18–20]. In contrast, CLIA demonstrated higher sensitivity, with values of 64.83%, along with a negative predictive value of 61.94% and an accuracy of 76.96%, surpassing those of ELISA. Although ELISA showed a specificity of 97.65% and a positive predictive value of 97.92%, no significant difference was observed compared to CLIA. This is consistent with the findings of another Chinese study [21]. A good correlation was found in pMN subjects between PLA2R antibody levels measured by CLIA and ELISA ($r = 0.674$, $P < 0.01$). When analyzing the coincidence rates, both CLIA and ELISA exhibited high levels of positive coincidence (96.55%), negative coincidence (91.61%), and total coincidence (93.35%). The Kappa value of 0.864 further indicated a strong consistency and coincidence between CLIA and ELISA in detecting anti-PLA2R antibody. In a study by Cornelia Dähnrich, CLIA demonstrated a higher sensitivity (83.9%) than ELISA (73.5%), while the specificity of both methods was similar (99.4%) [22]. Via ROC curve analysis, we found the AUC value of ELISA was 0.8737 ($P < 0.0001$), slightly lower than CLIA (0.8914, $P < 0.0001$). But the difference was not statistically significant ($P > 0.05$), meaning CLIA and ELISA have the same accuracy in diagnosis of pMN. We also get that the AUC of anti-PLA2R antibody measured by ELISA or CLIA was higher than that of serum albumin, total cholesterol, triglycerides, or 24-h urinary proteins. Thus, these data once again demonstrate the importance of PLA2R antibody as an alternative diagnostic biomarker for pMN. In contrast to our study, the proportion of CLIA-positive results among pMN specimens that were negative by ELISA was 39.0% in their research. Similarly, Elion Hoxha et al. compared CLIA, recombinant cell-based indirect immunofluorescence assay (RC-IFA), and ELISA for detecting and analyzing anti-PLA2R antibody. They found that CLIA exhibited excellent diagnostic performance similar to RC-IFA and outperformed ELISA in the diagnosis of MN and early recognition of recurrence [23]. It is important to note that differences between studies may arise from factors such as ethnicity, geography, treatment protocols (e.g., use of immunosuppressants), variations in detection techniques (e.g., epitope exposure, cut-off values, and Ig subtypes detected), and methodological approaches employed in the studies. Overall, the results indicate that CLIA offers enhanced sensitivity and a less labor-intensive approach compared to ELISA for detecting anti-PLA2R antibody. Though CLIA has high sensitivity, high specificity, simple and fast, long optical signal, no external light, but it still has shortcomings, such as enzyme sensitivity and high cost and maintenance. ELISA, while complex to operate, is low in cost and is a good choice for remote and under-budget areas.

This study has several limitations that should be acknowledged. Firstly, the sample size included in the study was small, and it was

conducted at a single center, which may restrict the generalizability of the findings. Secondly, we did not include a healthy population or patients with secondary membranous nephropathy as controls. Thirdly, other MN-related antigens such as THSD7A, exotoxin 1/exotoxin 2(EXT1/EXT2), Nel-like type 1 molecule(NELL1) may play an important role in the diagnosis and development of MN [24, 25]. While their antibodies were not measured in this study.

In conclusion, the detection of anti-PLA2R antibodies provides a noninvasive approach for diagnosing pMN, monitoring treatment response, and predicting recurrence, particularly in cases where renal biopsy is contraindicated. CLIA offers several advantages over ELISA, including automation, simplicity, and time-saving. With its promising characteristics, CLIA is poised to become a valuable method for anti-PLA2R antibody detection and is expected to find widespread use in clinical practice. Further research and validation studies are warranted to establish its clinical utility and broaden its application in managing MN patients.

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CRediT authorship contribution statement

Xiaotao Ma: Methodology, Conceptualization, Project administration, Writing – original draft. **Ruiting Wang:** Writing – original draft, Formal analysis, Investigation, Software. **Linting Wei:** Data curation, Visualization. **Pengfei Liu:** Supervision, Writing – review & editing. **Lanmei Jing:** Resources, Investigation. **Jinghua Wang:** Validation. **Wei Dong:** Investigation. **Xuefei Tian:** Writing – review & editing, Supervision. **Rongguo Fu:** Writing – review & editing, Conceptualization, Funding acquisition, Resources, Supervision.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review of the manuscript entitled.

Data availability

The data that has been used is confidential.

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