REVIEW

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PLA2R autoantibodies, a multifaceted biomarker in nephrotic syndrome and membranous nephropathy

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ABSTRACT

The phospholipase A2 receptor antibody (PLA2R-Ab) test is a valuable first-line diagnostic tool for primary membranous nephropathy (MN), helping to identify PLA2R-related MN and potentially eliminating the need for a kidney biopsy in some individuals. By reducing the reliance on biopsies, the test streamlines diagnosis and improves patient care. However, determining the optimal PLA2R measurement method and cut-off is critical to maximizing the benefits of the test and minimizing any harms. A systematic review and meta-analysis were performed to evaluate serum- and urine-based biomarkers for distinguishing between PLA2R-related MN and non-PLA2R MN. Searches were conducted in databases including Medline, Embase, Cochrane Library, Scopus, Web of Science, International HTA Database and ClinicalTrials.gov. The methodology followed Cochrane-recommended guidelines for systematic reviews and meta-analyses, and the QUADAS-2 tool was utilized to assess the overall risk of bias. Ninety-one studies met the eligibility criteria for inclusion in the review. Of these, 38 studies reporting the accuracy of the PLA2R-Ab test using the EUROIMMUN enzyme-linked immunosorbent assay (ELISA) method and 27 using the EUROIMMUN immunofluorescence (IF) method were suitable for meta-analysis. The pooled sensitivity and specificity of EUROIMMUN ELISA at a cut-off value of 20 RU/mL were 0.64 [95% confidence interval (CI) 0.56–0.72] and 94.7% (95% CI 90.5–97.1%), respectively. The pooled sensitivity and specificity of EUROIMMUN IF at a threshold of 1:10 was 0.69 (95% CI 0.637–0.739) and 0.98 (95% CI 0.931–0.994), respectively. Risk of bias was higher for studies evaluating the IF compared with ELISA test. We also explored whether the timing of the index test had an impact on the pooled diagnostic accuracy results; no significant differences were found. By evaluating the specificity and sensitivity of EUROIMMUN ELISA PLA2R-Ab and IF, we demonstrate that at ELISA levels ≥20 RU/mL, alongside thorough secondary screening, a kidney biopsy may be unnecessary. However, lower or negative levels still warrant a biopsy.

Keywords: non-invasive biomarkers, PLA2R, primary membranous nephropathy, sensitivity and specificity

KEY LEARNING POINTS

What was known:

- Diagnosing membranous nephropathy (MN) involves differentiating between phospholipase A2 receptor (PLA2R)-related MN from non-PLA2R-related forms of MN and other nephrotic syndromes.
- Various tests for anti-PLA2R antibodies are available, but their clinical utility varies.
- While kidney biopsy remains the gold standard, it carries inherent risks.

This study adds:

- The systematic review indicates that a EUROIMMUN enzyme-linked immunosorbent assay (ELISA) anti-PLA2R antibody level of 20 RU/mL is highly specific for PLA2R-associated MN, potentially avoiding the need for a kidney biopsy, if secondary causes are adequately screened.

Potential impact:

- Adopting a 20 RU/mL threshold for the EUROIMMUN ELISA anti-PLA2R test can reduce the reliance on invasive kidney biopsies, streamlining the diagnostic process for MN and improving patient safety by minimizing procedure-related risks.
- Our study highlighted research gaps in the diagnostic accuracy of PLA2R in diabetics and patients with renal impairment, requiring further investigation.

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INTRODUCTION

Membranous nephropathy (MN) is not a specific diagnosis but a pattern of glomerular injury caused by various disease entities. It is an organ-specific autoimmune condition involving immune complex formation at the subepithelial region of the glomerulus and complement activation, leading to protein loss through the filtration barrier [1]. The discovery of phospholipase A2 receptor antibody (PLA2R-Ab) testing has shifted MN diagnosis from a primary and secondary classification to a more precise categorization based on antigenic triggers [2, 3]. In most cases, MN is an immunological condition associated with autoantibodies targeting the M-type phospholipase A2 receptor (PLA2R1) on podocytes, with no link to other conditions. However, some cases are secondary to cancer, viral infections, systemic autoimmune diseases or medications. These are less likely to involve PLA2R-Abs and may involve other non-PLA2R-Abs [3–6].

Identifying PLA2R-Abs can aid MN management, provided accurate and reliable testing methods are used. Several testing methods, including western blot, enzyme-linked immunosorbent assay (ELISA) and chemiluminescence immunoassays, have been explored, each with unique advantages. ELISA is widely used, but there is no consensus on the type of kit or threshold value for positivity, highlighting the need for standardization [7–12].

While kidney biopsy remains the gold standard for MN diagnosis, it carries risks such as bleeding and infection, especially in anticoagulated nephrotic patients. Many centres continue to include biopsies in the standard work-up for MN; however, their added value in PLA2R-positive cases remains uncertain. In contrast, biopsies are crucial for PLA2R-negative patients to diagnose other nephrotic syndromes or identify alternative underlying pathologies driving MN [3, 13–16].

The high specificity of PLA2R-Ab testing challenges the necessity of kidney biopsy for PLA2R-positive patients. However, a 10%–30% prevalence of PLA2R-Ab in secondary MN suggests a potential overlap of aetiologies, complicating the interpretation of test results [17–19]. The application of PLA2R testing in diabetic patients presents unique challenges compared with non-diabetic individuals, possibly due to potential false positivity caused by His peptide binding [20].

The impact of renal impairment on diagnostic accuracy remains unclear, including whether biopsies to assess chronicity affect outcomes.

This systematic review evaluates biomarkers to determine an antibody threshold that could eliminate the need for biopsy in PLA2R-associated MN.

MATERIALS AND METHODS

We conducted a systematic review and, where appropriate, meta-analysis of published studies evaluating the diagnostic accuracy of non-invasive serum- or urine-based biomarkers to assess whether PLA2R serum Ab positivity can negate the need for a renal biopsy in patients with a positive test. The review methods and analysis followed the Cochrane Handbook for Systematic Reviews of Diagnostic Accuracy (version 2.0, 2023) [21]. The methodology was specified prior to conducting the review and registered in the PROSPERO International prospective register of systematic reviews (CRD42022304690) on 19 January 2022. The study is reported following Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Diagnostic Test Accuracy checklist recommendations [22].

Study selection

The study eligibility criteria used to determine study inclusion and exclusion can be found in Table 1.

Search strategy and screening

A comprehensive search (Supplementary data, Table S1) was conducted on 11 February 2022. The search was checked and rerun in

Table 1: Study eligibility criteria.

	Inclusion criteria	Exclusion criteria
Types of studies/ publications	Cohort studies and case–control studies	Case reports, literature reviews, editorials, letters, opinion pieces and non-peer-reviewed studies, including conference abstracts and posters Non-English language studies
Patient population	Adults aged 18 years or more, presenting with suspected or confirmed MN, defined as exhibiting proteinuria or nephrotic syndrome Studies had to include patient cohorts reported as primary MN and a comparator (e.g. secondary MN, other nephropathy or healthy controls)	Studies focused on paediatric populations or post-transplant populations
Target condition	MN	
Index test	We included all non-invasive biomarkers	Invasive diagnostic tests (e.g. biopsy-based tests such as the PLA2R1 stain test)
Reference standard	Renal biopsy (standard care)	
Outcome data		Studies where outcomes for MN were not reported separately Studies that did not report (directly or indirectly) 2 × 2 data (number of true positives, true negatives, false positives, and false negatives) for participants with MN
Timing	The index test must be conducted prior to or alongside renal biopsy to assess its effectiveness in diagnosing MN	Studies focused on index tests used in the context of prognosis for kidney disease progression

full on 2 May 2023 and 15 September 2024 to update the results through the end of 2024.

The following databases were searched: Embase, Medline, Scopus, Web of Science, the Cochrane Central Register of Controlled Trials and the Cochrane Database of Systematic Reviews. Unpublished (grey) literature was retrieved from the Clinical Trials Registry (ClinicalTrials.gov) and the International HTA Database (INAHTA, https://database.inahta.org/). The search terms combined key concepts pertaining to (i) membranous nephropathy and (ii) a series of named and generic biomarker terms, using subject headings and free text words. Limits for language and publication date were not used. The search was developed and run by an Information Specialist (N.K.) and peer-reviewed by a second Information Specialist using the Peer Review of Electronic Search Strategies (PRESS) checklist [23]. The search results were managed in an EndNote library to remove duplicates.

Screening was conducted in two stages. Identified records were first screened by title and abstract, with any studies expected to potentially meet the eligibility criteria included for further review. The full text of included records were then screened against the inclusion and exclusion criteria. The screening process was conducted in Rayyan (https://www.rayyan.ai/) [24].

The screening team consisted of four reviewers, with all records screened independently by two reviewers (either A.S. and O.R., or D.A.K.K. and P.H.) at both stages. To standardize the initial title and abstract screen, 200 records were randomly selected and independently co-screened by all four researchers; the resulting decisions were discussed to ensure consistency across the team. Any disagreements regarding the ultimate inclusion of a study were discussed with the full review screening team, and inclusion was determined by consensus.

Data extraction

The data extraction team consisted of five reviewers (A.S., D.A.K.K., O.R., P.H. and W.A.) using a data extraction form developed in Excel. To standardize the data extraction process, 10 studies were randomly selected and independently co-extracted by all five researchers, and the resulting decisions were discussed to ensure consistency across the team. Data were extracted on the following items:

- study details (e.g. authors, year, country, clinical setting)
- study methods (inclusion and exclusion criteria, patient selection methods, control group details)
- patient baseline characteristics (sex, age, data on kidney markers)
- test details [test(s) evaluated, control group(s) included, analytical method, in-house/commercial test details, diagnostic threshold(s), timing of test vs biopsy, whether any patients had initiated immunosuppression therapy]
- test accuracy data (number of true-positive, false-negative, true-negative and false-positive cases)

Each study was extracted by a single reviewer (O.R. or W.A.), with 20% independently extracted by a second reviewer (A.S., D.A.K.K. and P.H.). All accuracy data (i.e. the 2 × 2 data and thresholds) were checked by a second reviewer (A.S., D.A.K.K. and P.H.). Where 2 × 2 data was reported for different patient subgroups (e.g. using different controls), data for each subgroup was extracted.

Critical appraisal

Each included study was critically appraised using the QUADAS-2 tool [25] by a single reviewer (O.R. or W.A.) and checked by a second reviewer (A.S., D.K. and P.H.). Disagreements were resolved through discussion.

Statistical analysis

Meta-analysis was conducted, where appropriate, for studies that included a clinically relevant control group, which we defined as individuals with secondary MN and/or individuals with a range of diseases typically seen in the tested patient population such as other nephrotic syndromes/other glomerular diseases. Studies that only reported 2 × 2 data including healthy controls were excluded from the meta-analysis.

Meta-analysis was only appropriate/possible for studies reporting the accuracy of the PLA2R-Ab test using: (i) ELISAs from EUROIMMUN and (ii) EUROIMMUN immunofluorescence (IF). We planned to explore whether studies using different laboratory techniques, such as non-routine ELISA or other commercial or in-house methods produce different accuracy estimates, however, there was insufficient data to warrant meta-analysis. Where this data is available, the accuracy of each study is summarized in (Supplementary data, Tables S2–S5).

Many studies reported 2 × 2 tables at more than one threshold. A meta-analysis method for combining data at multiple thresholds across studies was used, allowing the estimation of pooled sensitivity and specificity at different thresholds [26]. Given specificity is of primary importance in this clinical context, we report accuracy at a range of thresholds for which specificity is greater than 80%. This analysis was conducted using the 'diagmeta' package in R [27]. Where studies reported accuracy at a single threshold, the bivariate model was used to pool sensitivity and specificity

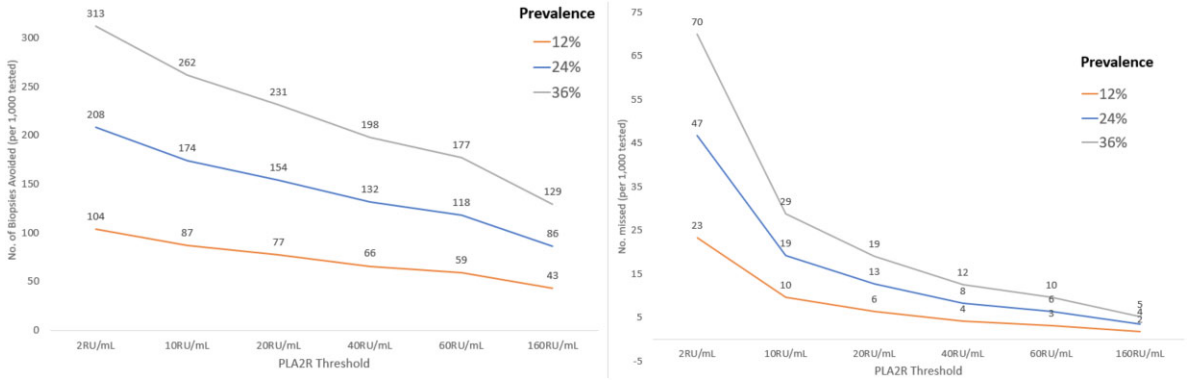

Figure 1: The number of biopsies that could be avoided (left) and the number of other glomerulonephritis causes including secondary membranous nephropathy missed (right) based on different PLA2R-Ab EUROIMMUN ELISA test thresholds and different prevalence levels.

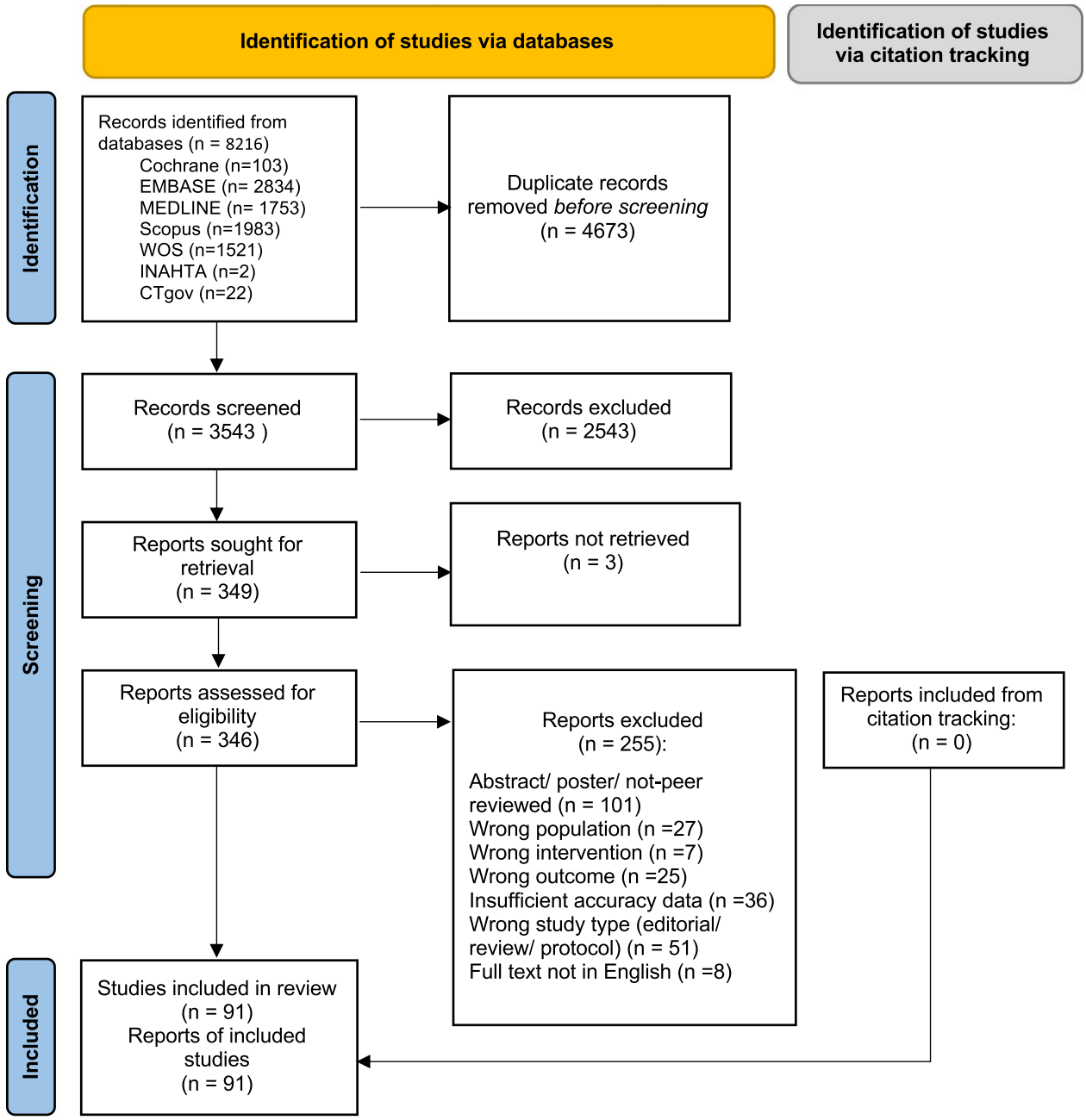

Figure 2: PRISMA flowchart describing study selection.

using the MetaDTA web app [28]. To help put the results in context, we also calculated the expected number of biopsies that could be avoided and the number of other glomerulonephritis causes including secondary membranous nephropathy missed based on different thresholds and prevalence levels (Fig. 1).

To calculate a representative serum creatinine value across all studies, we unified the reported values by approximating medians into means when interquartile ranges (IQRs) or ranges were provided. Values reported in mg/dL were converted to µmol/L using a standard conversion factor (1 mg/dL = 88.4 µmol/L). A weighted mean was then computed, with each study's creatinine value weighted by its sample size, providing a robust overall estimate that accounted for differences in reporting and study population sizes (Supplementary data, Fig. S4 and Table S9).

RESULTS

Search results and study characteristics of included studies

The search results at each screening phase and exclusions can be found in Fig. 2, with 91 studies ultimately included in the systematic review which included a total of 16 416 MN and non-MN patient. Characteristics of included studies are described in Table 2.

Risk of bias

Figure 3 provides a summary of the quality appraisal results using the QUADAS-2 tool [reported by study in Supplementary data, Fig. S1 and split by EUROIMMUN ELISA and IF testing in Supplementary data, Figs S2 and S3]. Nearly half of the studies

Table 2: Included study characteristics.

Number of studies	Total	91
	PLA2R-Ab studies	75
	Other biomarker studies	16
Year of publication	2009–24	
Study region	Asia	62
	Europe	15
	Africa	2
	North America	2
	Latin America	3
	Australia	3
	Not reported	4
Any funding conflicts of interest acknowledged	Yes	31
	No	20
	Not reported	40
Clinical setting	Hospital	78
	Nephrology centre	3
	Outpatient clinic	3
	Not reported	7
PLA2R-Ab tests	Total	101
	ELISA (any)	54
	EUROIMMUN ELISA methods	46
	Non-standard ELISA methods	4
	Non-reported ELISA methods	2
	Non-routine ELISA methods	2
	IF (any)	32
	Routine IF testing	31
	EUROIMMUN IF	27
	Non-routine IF testing	3
	Non reported IF method	1
	WB	8
	TRFIA	3
	QD-ICA	1
	ALBIA	1
	ChLIA	2
	Method not reported	1
Other tests reported	Non PLA2R-Ab methods	14
	THSD7A	8
	Other tests	6
Patient selection	Prospective	11
	Retrospective	22
	Prospective and retrospective	3
	Not reported	55
Reference group	Disease controls	65
	Healthy controls	11
	Disease controls + healthy controls	24

WB, western blot; TRFIA, time-resolved fluorescence immunoassay; QD-ICA, quantum dot-based immunochromatographic assay; ALBIA, addressable laser bead immunoassay; ChLIA, chemiluminescence immunoassay; THSD7A, thrombospondin type-1 domain-containing 7A.

were at high or unclear risk of bias in terms of patient selection; 35 studies included healthy controls in their patient sample and therefore were at high risk of overestimating diagnostic accuracy and many failed to report their patient selection methods in sufficient detail. There was also a concern about the applicability of the patient selection in a considerable proportion of the included studies. Reporting issues also impacted the ability to judge risk of bias for the index test and flow and timing domains, results in a considerable proportion consequently at an unclear risk of bias, as many studies did not report the time between index test and biopsy and not recording the immunosuppression status of their patients.

Diagnostic accuracy

PLA2R-Ab EUROIMMUN ELISA methods

Diagnostic accuracy of the PLA2R-Ab test using the ELISA EUROIMMUN method and an appropriate control was reported in 38 of the included studies [29, 9, 11, 30–64]. We extracted 64 distinct 2 × 2 tables from these studies, reporting accuracy across 16 different positivity thresholds. One additional study used both ELISA and IF and was not included [65].

Figure 4 shows the 2 × 2 estimates obtained from the studies. The colours represent the commonalities in the threshold. The curve represents the summary receiver operating characteristic (ROC); the area under the summary curve (AuSROC) is 0.91 (0.84; 0.94). The hatched area represents the confidence region for the estimated summary ROC curve, regions of the curve outside of the hatched area are based on a small number of studies or extrapolations and therefore cannot be interpreted with confidence. In Fig. 1, we present the number of biopsies that could be avoided and the number of other glomerulonephritis causes including secondary MN missed based on these PLA2R-Ab EUROIMMUN ELISA test thresholds and different prevalence levels.

Two of the extracted 2 × 2 tables is notably different from the cluster of points in the ROC space (the dark blue and red points furthest from the curve). This data comes from a study published by Song *et al.* and Suthar *et al.* 2022 [39, 63]. The reason for this difference could not be identified, but we explored what impact this study had on the overall results and found that excluding them from the analysis did not notably change the AuSROC 0.91 [95% confidence interval (CI) 0.83–0.95]. Many of the data points cluster at the top of the specificity axis. To explore whether the risk of bias relating to patient selection potentially influenced the results, we restricted the analysis to studies ($n = 6$) rated at low risk of bias for the patient selection domain of QUADAS-2. The AuSROC dropped only slightly to 0.90 (95% CI 0.80–0.94).

Of these studies, 21 conducted the PLA2R-Ab test before or at the same time as the biopsy, 3 studies allowed for a time interval longer than 6 months and 14 did not report the time interval between the PLA2R-Ab test and the biopsy (some studies reported 2 × 2 broken down into different time intervals). Restricting the meta-analysis to studies performing the PLA2R-Ab test before or at the same time as the biopsy ($n = 21$) resulted in an AuSROC of 0.90 (95% CI 0.80–0.94).

The diagnostic accuracy reported in the 6 additional studies using either an in-house ELISA method [30, 66, 67], a combination of EUROIMMUN and an in-house method [29] or another commercial method [68, 69] can be found in Supplementary data, Table S2. All study estimates were reasonably consistent in terms of their specificity (all were >94%) but report wide ranges of sensitivities (from 26% to 92%). The data available were insufficient to draw any conclusions about the accuracy of these different methods.

PLA2R-Ab IF methods

Diagnostic accuracy of the PLA2R-Ab test using the IF EUROIMMUN method, and an appropriate control was reported in 27 of the included studies [8, 31–33, 38, 40, 45, 51, 68, 70–74, 37, 75–86]. Six did not report the threshold for accuracy and had to be excluded from the meta-analysis [31–33, 70, 71, 87].

Nineteen studies reported accuracy at a single threshold (1:10). Only one study reported accuracy at other additional thresholds (1:100 and 1:1000) and one reported accuracy at 1:1000 (Supplementary data, Table S1). Due to insufficient data, we restricted the meta-analysis to the studies reporting accuracy at

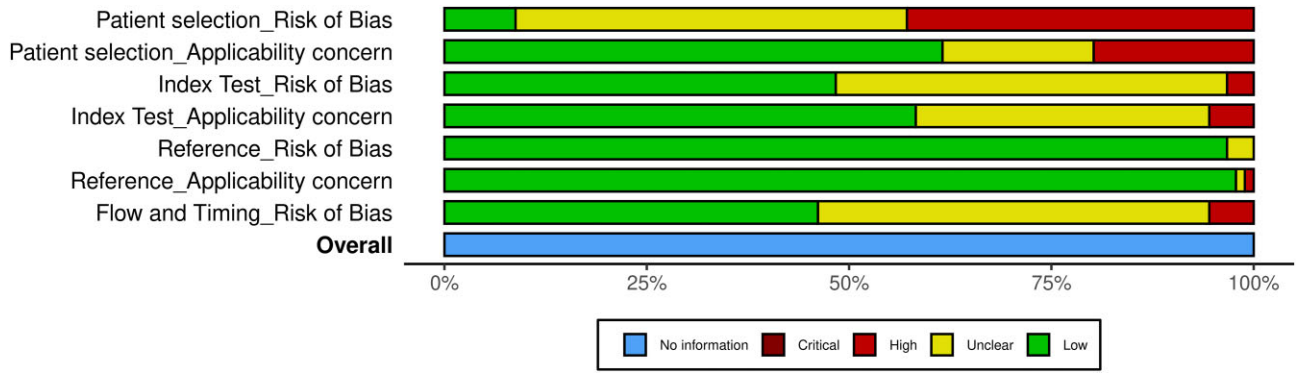

Figure 3: Summary of the risk of bias and applicability ratings using the QUADAS-2 critical appraisal tool for diagnostic accuracy studies.

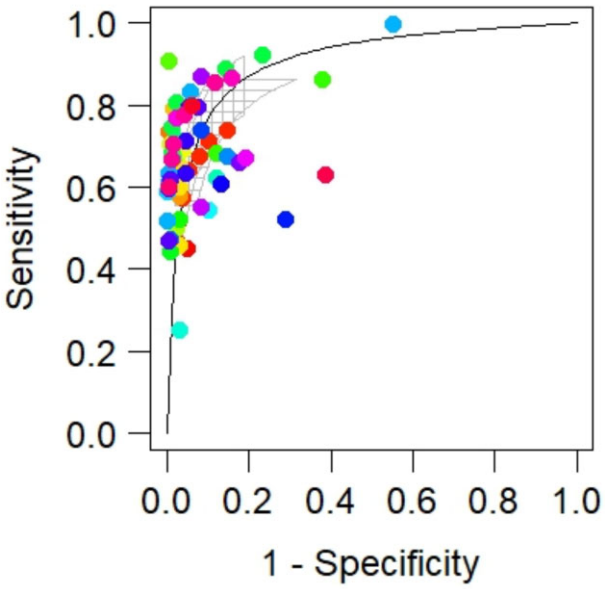

Figure 4: Multiple threshold summary ROC curve for PLA2R-Ab using the EUROIMMUN ELISA method.

1:10 ($n = 16$). The pooled sensitivity and specificity for the PLA2R-Ab test using the EUROIMMUN IF method at a threshold of 1:10 was 0.690 (95% CI 0.637–0.739) and 0.979 (95% CI 0.931–0.994), respectively (Fig. 5). Figure 5 shows the study estimates in ROC space and the pooled estimate; the size of the points illustrates the percentage weight of each study. The confidence region shows a fair amount of uncertainty in the pooled specificity and sensitivity estimates, which is also evident from the spread of the data points in the ROC space. The data points are coloured based on whether the study was rated at a high, low or unclear risk of bias for the index test domain of the QUADAS-2 appraisal. Studies at low risk of bias (highlighted in green) generally report lower specificities, therefore caution must be taken when interpreting this pooled estimate. Limiting the analysis to the five studies at a low risk of bias for the index test domain though did not result in a significantly lower pooled sensitivity (0.740, 95% CI 0.688–0.787) or specificity (0.974, 95% CI 0.874–0.995).

All our included studies reported serum testing apart from one reporting urine testing, which shows a high urine PLA2R specificity and sensitivity of 100% and 67.8%, respectively, using the IF test [87].

False positives

From 91 studies in our systematic review, 52 included comprehensive secondary screening (e.g. cancer, autoimmune and virology tests; Supplementary data, Table S7). Among these, 17 studies reported false-positive PLA2R-Ab results in 117 of 1015 patients (false-positive rate ~11%; Supplementary data, Table S8).

The majority of these false-positive cases were associated with secondary MN causes rather than other glomerulonephritides. Hepatitis B virus (HBV) was the most frequently reported secondary cause, identified in seven studies [66, 71, 35, 37, 75, 76, 88] followed by systemic lupus erythematosus (SLE) in five studies [35, 40, 75, 76, 89] and malignancy in three studies [38, 75, 70]. Among the studies using ELISA, PLA2R-Ab titres exceeding 20 RU/mL were observed exclusively in cases of HBV and malignancies [37, 38, 40, 61] Another study reported false-positive ELISA results at a cut-off >20 RU/mL in cases of SLE, minimal change disease, immunoglobulin A with subacute tubulointerstitial nephritis (TIN), and diabetic nephropathy with subacute TIN [61].

Other non-invasive biomarkers

We identified 16 studies using non-EUROIMMUN ELISA/immunofluorescence assay (IFA) for PLA2R-Ab and 14 using non-PLA2R methods, with no unified cut-off or standardized approach for conclusions (Supplementary data, Tables S2–S5).

DISCUSSION

This systematic review summarizes the evidence on serum PLA2R-Ab and other non-invasive biomarkers for MN, aiming to identify a reliable threshold to diagnose PLA2R-driven MN and distinguish it from other nephrotic syndromes/non PLA2R driven MN, potentially reducing the need for biopsies. A EUROIMMUN ELISA cut-off of 20 RU/mL was identified as the most acceptable threshold, with a pooled specificity of 0.95 (95% CI 0.91–0.97). The false-positive rate was low, mainly linked to HBV, SLE and cancers, detectable through routine screening. Adding EUROIMMUN IF enhances sensitivity with minimal improvement in specificity and may be useful in specific cases, such as diabetic patients.

A total of 16 416 patients with MN and non-MN were included in this systematic review. Here we have employed the latest recommended diagnostic accuracy meta-analysis methods to maximize the use of available data [26]. This method also estimates the threshold for specific pooled sensitivities and specificities, allowing us to explore pooled accuracy across different thresholds. This method was not implemented by previous studies [90–94]. We also refrained from applying a 'diagnostic accuracy' search

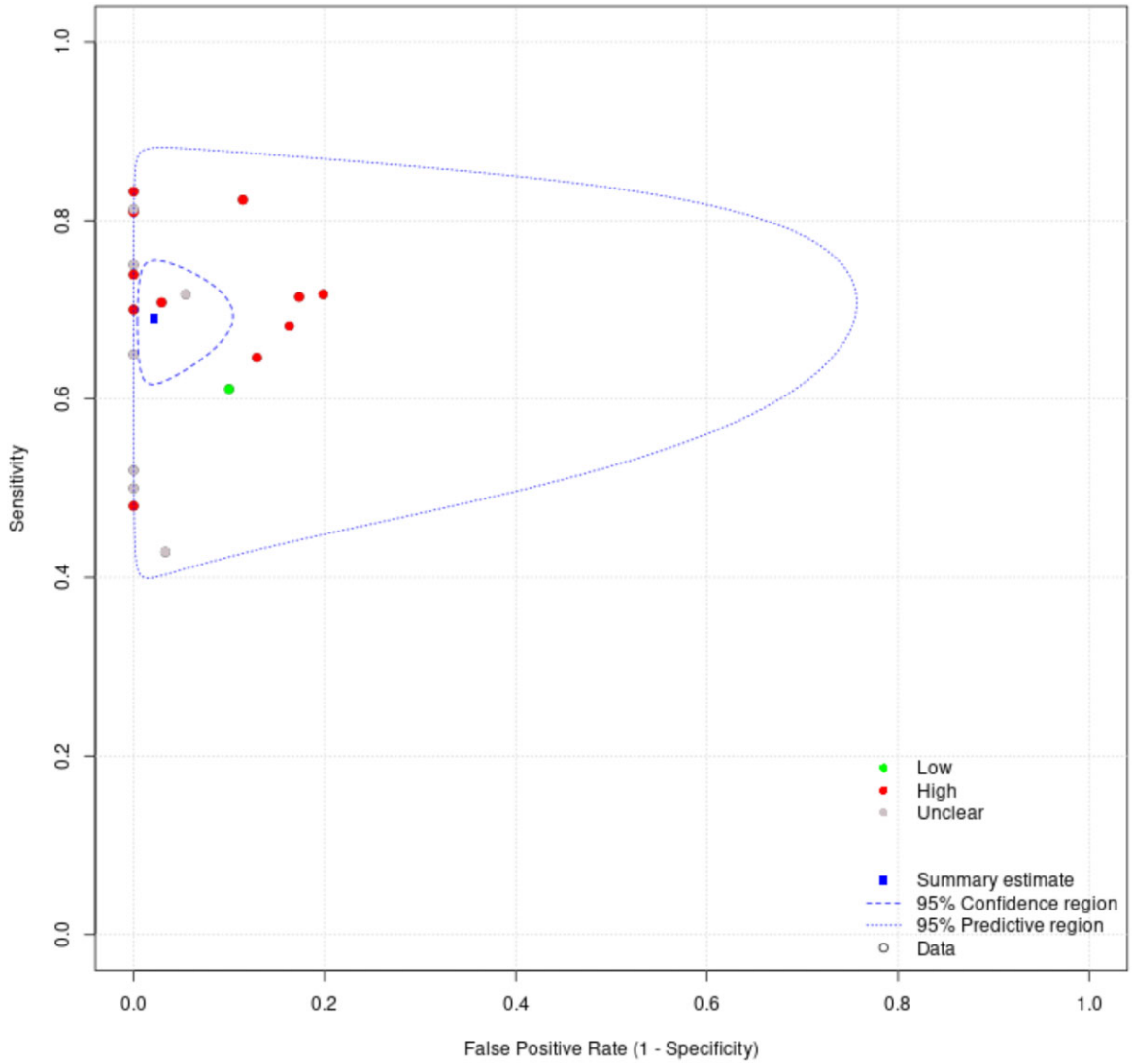

Figure 5: Bivariate meta-analysis of studies reporting the diagnostic accuracy of the PLA2R-Ab test using the IF EUROIMMUN method and a threshold of 1:10.

Table 3: Thresholds and pooled sensitivities and specificities extracted from the estimated summary ROC for the EUROIMMUN ELISA method.

Threshold (RU/mL)	All studies ($n = 38$)	
	Pooled sensitivity (95% CI)	Pooled specificity (95% CI)
2	86.8% (95% CI 78–92.4%)	80.5% (95% CI 66.8–89.5%)
10	72.7% (95% CI 64.6–79.5%)	92% (95% CI 86.5–95.4%)
20	64.3% (95% CI 55.8–72%)	94.7% (95% CI 90.5–97.1%)
40	54.9% (95% CI 44.9–64.5%)	96.5% (95% CI 93.1–98.3%)
60	49.2% (95% CI 38.1–60.4%)	97.3% (95% CI 94.2–98.8%)
160	35.8% (95% CI 23.1–51%)	98.5% (95% CI 96.1–99.5%)

filter to avoid missing key publications that might not have been found by other reviews [95, 96]. We included ELISA/IF studies, excluded western blot studies, and found no timing-related differences in PLA2R-Ab diagnostic accuracy.

False-positive anti-PLA2R ELISA results are rare but can occur in three key scenarios. First, non-specific binding may arise from factors such as immunoglobulin G (IgG) reacting to recombinant protein tags (e.g. His-tag) [20], surface antigens (e.g. bovine proteins) or IgG paraproteins/Rheumatoid Factor in serum, even when proper controls are applied. Second, individuals in a preclinical MN state may test positive for serum anti-PLA2R without proteinuria due to differing timelines of antibody production and disease pathology [97]. Regular monitoring, including IFA confirmation and genetic testing for DQ2 and PLA2R alleles, is advised. Third, non-MN pathologies, such as diabetes, may show false positives by ELISA but test negative on IFA [98]. Proper laboratory controls are critical to ensure high specificity for IMN diagnosis by EUROIMMUN ELISA.

At a threshold of 20 RU/mL for the EUROIMMUN ELISA PLA2R-Ab test and based on a prevalence of 24%, we estimated a pooled specificity of 94.7% (95% CI 90.5–97.1%), avoiding 154 kidney biopsies if 1000 individuals were tested as part of current standard practice [99]. Once a threshold of 60 RU/mL or higher is reached using EUROIMMUN ELISA, the degree of certainty in diagnosing a PLA2R MN is close to 100%, suggesting that a biopsy is unlikely to provide any new information and therefore exposing a patient to unnecessary risk (Tables 2 and 3). Radice *et al.* identified seven cases of cancer-associated MN confirmed by biopsy. The serum of all the samples tested positive for anti PLA2R-Ab, but none of

the renal biopsies could help identify a malignancy as the cause of the MN diagnosis [70]. Lefaucheur *et al.* used a cut-off of eight cells per glomerulus to distinguish malignancy-related MN cases from controls and noted a specificity of 92%, which was slightly lower than the pooled specificity of EUROIMMUN ELISA value of 20 RU/mL reported in our systematic review [100].

This raises the question of whether these findings are coincidental or are causally linked, as MN and malignancy often coexist due to age overlap. The Mayo Clinic consensus emphasizes classifying MN by underlying antigens, such as PLA2R, NELL1 or THSD7A, to guide diagnosis and treatment [3].

Dual positivity for PLA2R and other antibodies is rare. A positive PLA2R test indicates PLA2R-driven pathology, even when secondary causes like cancer and HBV contribute to antibody formation. Routine screening detects these conditions, often making biopsy unnecessary for further diagnosis.

Although the pooled specificity of the EUROIMMUN IF test in our review was 99%, using a quantitative marker like ELISA would better predict treatment response [101], especially given the higher risk of bias in studies evaluating EUROIMMUN IF. Few studies have reported that combining IF with ELISA enhances diagnostic accuracy [18, 102, 103]. Only one study in our analysis reported the diagnostic accuracy of combining PLA2R ELISA and IF testing; however, the testing method and cut off were unclear and they did not use the more common EUROIMMUN platform, making it challenging to draw conclusions or provide recommendations for clinical practice [65].

The combination approach could be particularly useful in specific scenarios to enhance diagnostic accuracy and address potential limitations of either test alone. One such scenario is in diabetic patients, where non-specific binding to components such as the His tag attached to recombinant PLA2R protein in ELISA assays can lead to false-positive results [20]. In these cases, the addition of IF can help confirm true PLA2R positivity and avoid diagnostic errors. Only two of the 91 included studies were adequately powered to evaluate the diagnostic accuracy of PLA2R in diabetic patients. While neither reported false-positive results, it is important to note that one utilized the EUROIMMUN ELISA at our recommended cut-off, while the other used the Oumeng ELISA method [36, 104].

Another scenario involves cases where detected PLA2R levels do not correspond with the clinical presentation, occurring years before proteinuria [105]. This discrepancy may stem from variations in PLA2R-Ab production, avidity or differing disease timelines [2]. In such challenging or atypical cases, we recommend the combined use of ELISA and IF to ensure a more comprehensive and accurate diagnostic approach.

Among 37 studies using EUROIMMUN ELISA/IF and reporting serum creatinine, the weighted mean was 82.14 µmol/L (Supplementary data, Table S9 and Fig. S4). Four studies with the highest levels reported two false positives (one unrecorded, one focal segmental glomerulosclerosis) out of 243 patients, with specificities ranging from 96.6% to 100% [59, 60, 68, 85]. Limited data on PLA2R serum test specificity in impaired renal function underscores the need for individualized biopsy decisions and further research.

Limitation

A key limitation of our study is the geographical imbalance, with most reports from Asia, though some representation from Europe and other Western regions was included. Additionally, insufficient data on studies reporting high serum creatinine in the EUROIMMUN ELISA/IF groups and on patients presenting with diabetes precluded a subgroup meta-analysis.

CONCLUSION

The PLA2R-Ab test using the EUROIMMUN methods (ELISA/IF) is highly specific for diagnosing PLA2R-related MN, distinguishing it from other nephrotic syndromes and non-PLA2R MN. The EUROIMMUN ELISA value ≥20 RU/mL, combined with secondary screening, can confirm PLA2R-related MN without requiring a biopsy. For patients with impaired renal function or diabetes, decisions should be made on a case-by-case basis.

SUPPLEMENTARY DATA

Supplementary data are available at *Nephrology Dialysis Transplantation* online.

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AUTHORS' CONTRIBUTIONS

O.R., D.A.K.K., A.S. and P.H. conceptualized the study; O.R., D.A.K.K., W.A., A.S. and P.H. were responsible for review screening and extraction processes; O.R., W.A. and A.S. were responsible for data curation; B.S. conducted the meta-analyses; N.K. was responsible for designing and running the search strategies; O.R. wrote the original draft; P.B., D.A.K.K., W.A., B.S., J.B. and P.H. reviewed and edited the manuscript.

DATA AVAILABILITY STATEMENT

The data underlying this article are available in the article and in its online supplementary material.

CONFLICT OF INTEREST STATEMENT

None declared.

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