REVIEW ARTICLE

Anti-phospholipase A2 receptor antibodies: Current applications in primary membranous nephropathy

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■ ABSTRACT

Background: Membranous nephropathy (MN) is among the most common causes of nephrotic syndrome in nondiabetic adults. The podocytic M-type phospholipase A2 receptor (PLA2R) has been identified as the major antigenic target of the immune response that underlies primary membranous nephropathy (PMN). In the last decade, anti-PLA2R antibodies have been seen as promising diagnostic biomarkers for PMN. Other potential uses include assessment of disease activity and prognosis, therapeutic monitoring and prediction of disease recurrence after renal transplant. **Objective**: To review clinical studies exploring the current role of anti-PLA2R antibodies. **Methods**: This systematic review was conducted according to PRISMA guidelines. Two databases were searched for articles published between January 2010 and August 2019. Fifty-one studies met the inclusion criteria. **Results**: Anti-PLA2R antibodies constitute highly sensitive and specific markers for PMN. Antibody titers correlate positively with disease activity. Low levels or seronegativity correlate to higher remission rates. Relative reduction of antibody titers seems to correlate with the likelihood of response to therapy. The value of this antibody in the prediction of post-transplant recurrence is uncertain, as is its use in the pediatric population. **Conclusion**: Anti-PLA2R antibodies are assuming an increasingly important role in MN management. Anti-PLA2R positivity in the context of nephrotic syndrome is very suggestive of PMN diagnosis. Nevertheless, further efforts are required to define optimal cut-off values for seropositivity and risk stratification.

Keywords: Anti-PLA2R; Biomarkers; Membranous nephropathy.

■ INTRODUCTION

Membranous nephropathy (MN) is the denomination of a specific glomerular disease induced by the deposition of immune complexes at the glomerular basement membrane (GBM), causing its thickening.[1] This immunological process alters the permeability of the glomerular vasculature and is the most common etiology of nephrotic syndrome (NS) in Caucasian adults.[2]

MN's long-term outcomes have been said to follow the "rule of thirds". According to this model, one third of diagnosed patients evolve towards spontaneous remission, one third maintain persistent proteinuria, while another third develop end-stage renal failure in ten years.[3] Currently, this depiction is considered excessively simplistic and most clinicians opt for a risk-stratification approach when predicting a patient's prognosis and determining the optimal treatment strategy.[4]

MN is classically divided into two subgroups: primary MN (PMN) and secondary MN (SMN). SMN constitutes around 20% of all MN cases and occurs in the context of underlying conditions (malignancies, drugs, rheumatological diseases and infections).[5] PMN refers to the remaining cases with no identifiable underlying cause. The pathological mechanism of PMN still remains to be clarified.[6]

In 1959, *W. Heymann* developed an experimental model in rats that identified megalin, a podocyte antigen, as the main target for circulating antibodies responsible for the formation of GBM deposits.

Despite the importance of this discovery, the mechanism described wasn't directly transposable to humans.[7-11]

In 2003, *P. Ronco* observed that pregnant women with neutral endopeptidase (NEP) deficiency developed IgG1 and IgG4 NEP-binding antibodies capable of crossing the placenta, inducing neonatal MN. This confirmed that podocyte antigens constitute potential targets for circulating antibodies that may lead to complement activation and subsequent deposition of glomerular immune complexes.[12,13]

In PMN specifically, M-type phospholipase A2 receptor (PLA2R) has been identified as the main target antigen. Circulating anti-PLA2R antibodies are present in approximately 70% of PMN patients.[14] Other antigenic targets include thrombospondin type 1 domain–containing 7A (THSD7A), with anti-THSD7A antibodies present in 3–5% of PMN patients.[15,16] Recently, using laser microdissection of glomeruli and mass spectrometry analysis of renal biopsies, *Sethi et al.* discovered two novel antigens that also play a role in MN: exostosin (associated with autoimmune disorders) and NELL-1.[17-19]

Recent studies suggest a strong correlation between the levels of circulating anti-PLA2R antibodies and disease activity, typically estimated through proteinuria quantification. Special care is required in the interpretation of discordant antibody and proteinuria levels. Low or absent antibody levels may indicate remission or decreased disease activity, but simultaneously increased proteinuria levels may constitute an indirect sign of glomerular structural changes and tubular damage.[20]

Studies also suggest anti-PLA2R quantification may be used to predict disease outcomes and to evaluate patient response to treatment.[21,22] Other potential applications of this marker include the estimation of the risk of PMN recurrence after kidney transplant and immunosuppressive therapy optimization.[23-25]

In this paper, we analyzed data from recent clinical studies to understand the current role of anti-PLA2R antibodies on several fronts: MN diagnosis, determination of disease activity, therapeutic monitoring, differential diagnosis with secondary forms and evaluation of the risk of disease recurrence.

■ METHODS

This systematic review was conducted in accordance with Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The article search for the present review was carried out from September 2018 to September 2019.

Two different electronic databases were used, PUBMED and SCOPUS. The authors agreed on a set of inclusion and exclusion criteria for automatic depuration. The search criteria were coded into queries specific to each database with "phospholipase a2" and "membranous nephropathy" as keywords.

The resulting papers had their abstracts screened following pre-defined criteria to select original articles with well-defined assessment methodology relevant to the scope of this review.

Figure 1 attempts to summarize the article search and depuration protocol for this paper.

The following information – study type, subject number, anti-PLA2R positivity, PLA2R expression, assay used and clinical outcomes – was extracted for each selected paper (when applicable) and are shown on table I. Divergences over inclusion of studies and interpretation of data were resolved by consensus discussion. Due to significant heterogeneity in terms of methodology, participant selection, and reported outcome measures, the results of the included studies were not pooled but instead presented qualitatively.

■ RESULTS

In total, 51 articles were critically analyzed. Most of the selected studies were prospective in nature (n = 31, 60.8%). All patients with MN included in the clinical trials had pathological confirmation of their diagnosis. Anti-PLA2R antibody detection was predominantly performed through conventional enzyme-linked immunosorbent assay (ELISA) and indirect immunofluorescence testing (PLA2R IIFT, Euroimmun).

Figure 1

Breakdown of obtained manuscripts from the initial search to studies included in the review, including queries used for each database.
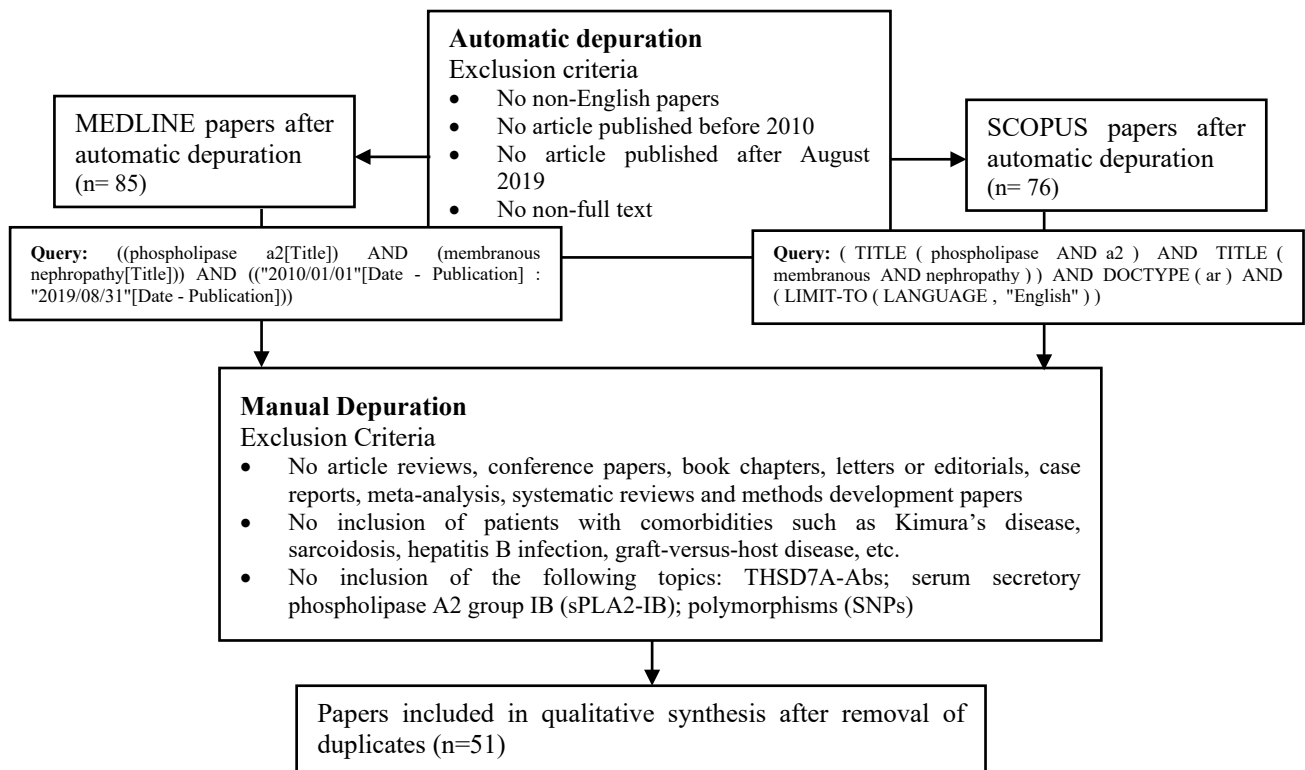

Automatic depuration
Exclusion criteria
- No non-English papers
- No article published before 2010
- No article published after August 2019
- No non-full text

MEDLINE papers after automatic depuration (n= 85)

Query: ((phospholipase a2[Title]) AND (membranous nephropathy[Title])) AND (("2010/01/01"[Date - Publication] : "2019/08/31"[Date - Publication]))

SCOPUS papers after automatic depuration (n= 76)

Query: (TITLE (phospholipase AND a2) AND TITLE (membranous AND nephropathy)) AND DOCTYPE (ar) AND (LIMIT-TO (LANGUAGE , "English"))

Manual Depuration
Exclusion Criteria
- No article reviews, conference papers, book chapters, letters or editorials, case reports, meta-analysis, systematic reviews and methods development papers
- No inclusion of patients with comorbidities such as Kimura's disease, sarcoidosis, hepatitis B infection, graft-versus-host disease, etc.
- No inclusion of the following topics: THSD7A-Abs; serum secretory phospholipase A2 group IB (sPLA2-IB); polymorphisms (SNPs)

Papers included in qualitative synthesis after removal of duplicates (n=51)

Table I

Author (year)	Study type	Patients (n)	Anti-PLA2R1+ (n/%)	PLA2R expression %	Assay	Clinical outcome
Hoxha et al., 2014[47]	Prospective	n = 133	NR	NR	ELISA IIFT	81% reduction in anti-PLA2R antibody levels paralleled by a 39% reduction in proteinuria.
Hoxha et al., 2014[43]	Prospective	n = 118	NR	NR	ELISA	High anti-PLA2R antibody levels are associated with an accelerated loss of renal function.
Hihara et al., 2016[34]	Prospective	n = 59 38 PMN / 21 SMN	19/50% PMN 0/0% SMN	NR	ELISA IIFA	Anti-PLA2R positivity was highly specific of PMN and was associated with glomerular IgG4-predominant deposition.
Qin et al., 2011[30]	Prospective	n = 106 60 PMN / 46 SMN	49/82% PMN 5/10.9% SMN	NR	WB	Detection of anti-PLA2R antibodies is a sensitive test (82%) for PMN. Anti-PLA2R positivity was largely absent in SMN patients.
Xu et al., 2017[55]	Retrospective	n = 231	NR	189/81.8%	IIFA	Non-PLA2R associated PMN responds more quickly to IS therapy. There were no significant differences in terms of renal function.
Ardalan et al., 2013[68]	Prospective	n = 30 23 PMN / 2 SMN	17/74% PMN 0/0% SMN	NR	IIFA	No correlation found between anti-PLA2R antibody and proteinuria level. Proteinuria is not a good marker of disease activity but may reflect the glomerular damage.
Gupta et al., 2016[60]	Retrospective	n = 16	NR	NR	ELISA	Pre-transplant PLA2RAb > 29 RU/mL predicts MN recurrence with a sensitivity of 85% and a specificity of 92%. Patients with recurrent disease despite undetectable anti-PLA2R antibodies should be studied for occult malignancy and different antigens.
Yang et al., 2016[35]	Retrospective	n = 438	NR	NR	ELISA	IgG4 levels have higher predictive accuracy for clinical remission than total antibodies.
Zhang et al., 2017[28]	Retrospective	n = 458	1st cut-off: 84.06% 2nd cut-off: 71.01%	NR	TRFIA	Serum anti-PLA2R-IgG levels are significantly higher in PMN patients than in those with SMN. Renal deterioration is more severe in patients with higher antibody titers.
Kim et. al., 2015[26]	Prospective	n = 160 93 PMN / 14 SMN	41/44.1%	NR	ELISA WB	High levels of anti-PLA2R antibodies are correlated with low remission rates and greater disease severity. ELISA and WB have a concordance rate of 72.1%.
Hayashi et al., 2015[58]	Prospective	n = 25	16/73%	NR	ELISA	PLA2R-related PMN patients have a lower remission rate and a worse clinical outcome than PLA2R-unrelated PMN patients. IgG4-dominant deposition greater in PLA2R related PMN.
Song et al., 2018[38]	Prospective	n = 66	NR/52.1%	NR	ELISA	Higher spontaneous remission rates in anti-PLA2R-negative patients. Positive correlation between proteinuria and anti-PLA2R antibody levels. Incidence of stage 3 and above CKD is higher in anti-PLA2R positive patients.
Jullien et al., 2017[50]	Retrospective	n = 68	NR	NR	ELISA	Anti-PLA2R antibodies vary in a dose-response manner with remission. Anti-PLA2R antibody baseline values are not useful to predict disease activity.
Radice et al. 2018[31]	Prospective	n = 479 178 PMN 32 SMN	70.6% PMN	NR	IIFA	Anti-PLA2R positivity in a MN patient is not sufficient to exclude a secondary cause, particularly in patients considered at risk for malignancies.
Jatem Escalante et al., 2015[49]	Retrospective	n = 55	NR	NR	ELISA	Anti-PLA2R antibody negativity is associated with higher likelihood of spontaneous remission.
Liu et al., 2018[27]	Prospective	n = 39 27 PMN 5 SMN	24/88.9% PMN 2/40% SMN	NR	ELISA	Serum anti-PLA2R antibody and PLA2R expression in kidney tissue had an 100% concordance rate.
Akiyama et al., 2015[48]	Retrospective	n = 131 100 PMN 31 SMN	53/53% PMN 0/0% SMN	NR	WB	The prevalence of anti-PLA2Rabs is higher in patients with NS when compared to patients without this condition. Positivity of anti-PLA2R is correlated with a greater number of patients with serum albumin ≤ 3g/dL.
Hoxha et al., 2015[36]	Prospective	n = 37	NR	NR	IIFA	The use of IS does not alter the probability of achieving remission in seronegative PMN patients.
Qin et al., 2016[46]	Retrospective	n = 572	392/68.5%	519/90%	ELISA IF	Patients who have dual positivity for anti-PLA2R antibodies and PLA2R glomerular antigen have higher levels of proteinuria and lower probability of achieving remission. Disease relapse was more frequent patients with higher levels of PLA2R antigen deposition.
Hoxha et al., 2012[45]	Prospective	n = 88	60/68%	61	IIFA	No correlation was found between proteinuria and anti-PLA2R levels. A strong correlation was found between antibody levels and glomerular expression of PLA2R antigen in PMN patients.
Medrano et al., 2015[53]	Prospective	n = 79	NR	NR	ELISA	No correlation was found between antibody levels before treatment and clinical outcome. Relative antibody titer reduction has a superior predictive value than absolute values at 3- and 6-months post-treatment.

Author (year)	Study type	Patients (n)	Anti-PLA2R1+ (n/%)	PLA2R expression %	Assay	Clinical outcome
Dou et al., 2016[32]	Prospective	n = 229	1st cut-off: 65.3% 2nd cut-off: 60.2% 3rd cut-off: 45.8%	NR	ELISA	A cutoff of 14 RU/ml should be used to diagnose PMN.
Ruggenenti et al., 2015[51]	Prospective	n = 132	NR	NR	ELISA	Anti-PLA2R antibody depletion preceded complete remission. Low anti-PLA2R titer at baseline and full antibody depletion 6 months post-rituximab predicted remission. Disease relapse was predicted by resurgence of antibodies.
Kumar et al., 2015[62]	Prospective	n = 5	3/60%	100%	ELISA	There seems to be a role for PLA2R in the pathogenesis of pediatric PMN, but more studies with larger samples are needed.
Seitz el al., 2014[59]	Retrospective	n = 15	NR	NR	ELISA	IgG4 anti-PLA2R positivity at the time of transplant doesn't predict recurrence. Positivity for IgG4 anti-PLA2R during follow-up (after 6 months) is a risk factor for recurrence. IgG4 anti-PLA2R titers may help predict disease relapse.
Roy et al., 2017[56]	Retrospective	n = 190	70.2%	NR	IHC	Anti-PLA2R IHC is a specific marker for differential diagnosis between PMN and SMN.
Seitz-Polski et al., 2018[67]	Prospective	n = 58	NR	NR	ELISA	Epitope spreading is an independent negative predictor of remission.
Wang et al. 2016[37]	Retrospective	n = 131	NR	NR	ELISA IIFA	There is a correlation between seronegativity and remission. High titers correlate to greater disease activity. Dual negativity for anti-PLA2R antibodies and glomerular PLA2R deposits suggest SMN.
Yachha et al., 2018[44]	Prospective	n = 63	NR/55.2% PMN 0/0% SMN	NR	ELISA	There appears to be a direct correlation between antibody titers and disease activity. Seropositive patients presented worse results in terms of hypoalbuminemia and proteinuria.
Segarra-Medrano et al. 2014[69]	Prospective	n = 64 47 PMN 17 SMN	IIFA – 72.3% PMN ELISA – 74.5% PMN	76.6%	IIFA ELISA IHC	No correlation was found between anti-PLA2R titers and proteinuria or albuminemia. IIFA and ELISA have high concordance rates and specificity. Seropositive patients with anti-PLA2R deposits have present with more significant proteinuria.
Liu et al., 2016[65]	Prospective	n = 252	NR	82% PMN 16.7% SMN	IIFA	Dual positivity can be useful to distinguish PMN from SMN. PMN patients with positivity for glomerular PLA2R deposits present superior response to IS.
Oh et al., 2013[39]	Prospective	n = 100	69/69%	NR	WB	Anti-PLA2R antibody levels correlate directly to proteinuria and hypoalbuminemia. No correlation was found between anti-PLA2R antibody levels and remission.
Debiec et al., 2011[23]	Retrospective	n = 19	NR	NR	IIFA WB	No correlation was found between anti-PLA2R antibody levels at the time of transplantation and disease recurrence.
Lin et al., 2015[29]	Retrospective	n = 279	NR/60%	NR	ELISA	Anti-PLA2R antibody levels correlate to proteinuria and hypoalbuminemia. Good diagnostic accuracy for PMN.
Gopalakrishnan et al., 2016[40]	Prospective	n = 75 60 PMN 15 SMN	45/75%	NR	IIFA	Anti-PLA2R antibody levels correlate to proteinuria and hypoalbuminemia.
Kimura et al., 2017[70]	Retrospective	n = 169	NR/48% PMN NR/0% SMN	NR	IIFA	Alfa enolase autoantibody may facilitate the access of other antibodies to the podocyte (ex. anti-PLA2R).
Kanda et al., 2017[61]	Retrospective	n = 34	NR	6%	SIM	The two patients with PLA2R positivity were over 10 years of age. Granular glomerular staining is weaker when compared to PMN adult patients. These findings suggest PLA2R has a limited role in pediatric PMN, particularly in preteen patients.
Wu et al., 2017[41]	Retrospective	n = 73	NR/85.4% PMN NR/29.4% SMN	91.7% PMN 29.4% SMN	IIFA	Anti-PLA2R antibody levels and glomerular PLA2R deposits correlate to proteinuria and hypoalbuminemia (disease activity).
Yeo et al., 2018[64]	Retrospective	n = 115	NR	83%	IHC	PLA2R and IgG4 bipositivity showed a specificity of 96.4% for PMN. This detection method can be useful to differentiate primary and second forms of MN.
Radice et al., 2016[42]	Prospective	n = 42	NR	NR	IIFA	Anti-PLA2R antibody levels correlate to proteinuria and hypoalbuminemia. The probability of proteinuria remission increased 6.5 times after seronegativation. Evaluation of anti-PLA2R may be useful for optimizing IS.
Segarra-Medrano et al., 2014[54]	Prospective	n = 36	NR	NR	ELISA	No correlation was found between antibody levels before treatment and clinical outcome. Relative reduction of antibodies level was significantly greater in those who achieved remission. Relative reduction in antibody titers at 3 months predicted response at 6 and 9 months. Relative titer reduction at 6 months predicted response at 12 months.
Kao et al., 2015 (66)	Prospective	n = 74	NR	NR	NR	A three-domain complex consisting of cysteine- rich (CIS), fibronectin-like type II (FnII) and C-type lectin-like domain 1 (CTLD1) is likely to be the immunodominant epitope of PLA2R.

Author (year)	Study type	Patients (n)	Anti-PLA2R1+ (n/%)	PLA2R expression %	Assay	Clinical outcome
Tampoia et al., 2018[33]	Prospective	n = 67	NR/ 88.1%	NR	ELISA	Anti-PLA2R antibody levels and glomerular PLA2R deposits correlate to proteinuria, hypoalbuminemia and serum creatinine.
Zhang et al., 2019 (63)	Retrospective	n = 225	NR	82.7% AP 42.1% PP	IHC	Clinical features and prevalence of PLA2R positive staining in adolescent patients with PMN was similar to the adult population, suggesting close etiology and pathogenesis. Younger children with PMN were mostly negative for PLA2R staining, suggesting different underlying etiology.
Barrett et al., 2019[3]	Prospective	n = 14	14/100%	NR	ELISA	Belimumab treatment in participants with PMN can reduce anti-PLA2R-antibody levels, subsequently reducing proteinuria.
Bobart et al., 2018[15]	Retrospective	n = 838 90 PMN	143/17.1%	96.7%	ELISA SIM	Among patients with preserved kidney function and no evidence of secondary causes, a positive PLA2R antibody test is highly predictive of tissue diagnosis of PLA2R-associated MN.
Cheng et al., 2018[71]	Retrospective	n = 259 146 PMN	102/69.9%	NR	ELISA	Study findings underscore the diagnostic potential of total serum anti-PLA2R antibodies and highlight the importance of antibody subtype 4 over other subtypes.
Chi et al., 2019[72]	Prospective	n = 79 72 PMN	50/69.4%	NR	ELISA IIFA	Indirect evidence of anti-PLA2R antibody as an etiologic factor in PMN patients. The role of C5a in the disease course and on inflammatory cells is worth clarifying.
Provatopoulou et al., 2018[73]	Retrospective	n = 33	16/48.5%	NR	ELISA	Low levels of anti-PLA2R antibodies at diagnosis are predictive of disease remission following IS. Serial anti-PLA2R measurements correlate with clinical status throughout the follow-up period.
Qu et al., 2018[52]	Prospective	n = 359	234/75.2%	NR	ELISA IIFA	Antibody positivity and antibody titers might predict treatment response and kidney outcomes in PMN.
Li et al., 2019[57]	Prospective	n = 51	34/66.77%	45/88.24%	ELISA SIM	Disease status and prognosis correlated more closely with serum Anti-PLA2R antibody titers than with the presence of glomerular PLA2R antigen deposits. Serum positivity was associated with more severe clinical symptoms and worse prognosis, which was probably associated with increased IgG4 deposition.
Fervenza et al., 2019[74]	Prospective	n = 130	96/74%	NR	ELISA	Rituximab was noninferior to cyclosporine in inducing complete or partial remission of proteinuria at 12 months and superior in maintaining proteinuria remission up to 24 months.

AP – Adult population; CKD – Chronic kidney disease; ELISA – Enzyme-linked immunosorbent assay; EM – Electron microscopy; PMN – Primary membranous nephropathy; IHC – Immunohistochemistry; IIFA – Indirect immunofluorescence assay; IS – Immunosuppression; NR – Not reported; NS – Nephrotic syndrome; PP – Pediatric population; SIM – Standard immunofluorescence microscopy; SMN – Secondary membranous nephropathy; SNP – Single nucleotide polymorphisms; TRFIA – Time-resolved fluoroimmunoassay

■ Anti-PLA2R antibodies and PMN diagnosis

The consensus is that anti-PLA2R antibodies constitute highly sensitive and specific biomarkers of PMN in the adult population.

Reported sensitivity varied between 44.1 and 88.9%.[26,27] Interstudy variability mainly relates to different cut-off values and quantification methods used. The inclusion of patients already under immunosuppressive treatment may have contributed to the emergence of false negatives.[28-34]

Regarding ELISA testing, commercially available kits have a manufacturer's recommended cut-off value of 14 RU/mL, used by most of the included articles. *Tampoia* et al. adopted an adapted cut-off value of 2.7 RU/mL with increased sensitivity (88,1%) and no impact on the test's specificity.[33]

■ Anti-PLA2R titers, disease activity and response to therapy

Most included studies suggested a linear correlation between antibody titers and PMN activity.[26,33,43-48,35-42]

Isolated pre-treatment values appear to be inaccurate predictors of response to treatment. Anti-PLA2R seronegative individuals, however, were found to have higher likelihood of spontaneous remission.[20,30,36-38,46,49-52] In these cases, *Hoxha et al.* reported that immunosuppression does not seem to increase the likelihood of remission when compared to supportive therapy.[36]

The rate of anti-PLA2R decline seems to be a superior indicator of response to treatment. Two studies by *Medrano et al.* showed no association between isolated antibody titers prior and clinical response. In the same trials, the relative reduction of antibodies after initiation of therapy had an apparent correlation with time elapsed until remission and the likelihood of achieving it.[53,54]

■ Anti-PLA2R antibody titers versus antigen deposits

Various authors also investigated the role of PLA2R antigen glomerular deposits as biomarkers of MN – despite reports of higher sensitivity when compared to anti-PLA2R antibodies, PLA2R antigen deposits seem to have a weaker correlation with disease status and prognosis. Dual positivity is correlated with higher disease activity

and lower remission rates. Dual negativity, on the other hand, is highly suggestive of secondary MN.[27,37,45,46,55-58]

■ Anti-PLA2R antibodies and renal transplantation

There is no consensus regarding the usefulness of anti-PLA2R baseline quantification when it comes to stratifying the risk of disease recurrence after kidney transplant. Two of the reviewed articles dismiss a correlation between antibody titers at the time of transplantation and risk of disease recurrence.[23,59] On the other hand, *Gutpta et al.*'s results suggest pre-transplant titers above 29RU/mL are predictive of disease recurrence with high sensitivity (85%) and specificity (92%).[60] *Seitz-Polski et al.* suggest that positive IgG4 anti-PLA2R activity during follow-up is a significant risk factor for MN relapse.[59]

■ PLA2R studies in the pediatric population

Only three clinical trials regarding pediatric patients were included in this review. In children, serum anti-PLA2R antibody and renal PLA2R antigen positivity is less frequent than in adult PMN patients. Adolescent patients with PMN, however, seem to exhibit similar rates of PLA2R+ staining to those found in adults.[61-63]

■ Anti-PLA2R antibodies and non-invasive PMN diagnosis

Bobart et al. reviewed the potential of anti-PLA2R testing for non--invasive diagnosis of PMN. In this study, anti-PLA2R quantification was prompted in most cases due to the presence of nephrotic range proteinuria or nephrotic syndrome. 143 patients tested positively. Of these, 132 were subjected to kidney biopsy (KB) that confirmed MN diagnosis. After exclusion of secondary causes, KB provided little or no management-altering information.[15]

■ DISCUSSION

This systematic review shows the persistently increasing value of PLA2R studies in MN patients, particularly when it comes to diagnosis and disease management. Despite our expanding grasp of the significance of anti-PLA2R antibodies, several areas still require further investigation.

One of these relates to ELISA cut-off values. As previously mentioned, reported sensitivity for anti-PLA2R ELISA testing had significant interstudy variability (44.1 to 88.9%). Reducing the threshold for positivity might push results higher up the sensitivity spectrum with no prejudice to test specificity as suggested by *Tampoia et al.*[33]. Further studies are required to validate these findings, but if results are consistent, the recommended cut-off value should be significantly reduced.

In recent years, several studies have confirmed that different immunoglobulin IgG subtypes relate to distinct MN etiologies – IgG4 is predominant in PMN, while IgG1 and IgG3 occur mainly in SMN.[27,34,58,64,65] *Yang et al.* suggest anti-PLA2R IgG4 monitoring might constitute a more effective technique than total antibody quantification.[35] Further research is necessary to better understand immunological interactions in PMN patients and to determine how IgG characterization would be better applied in our clinical practice.

As previously mentioned, anti-PLA2R baseline quantification in transplant recipients is of uncertain value. Of the three included articles on this topic, two dismiss a correlation between antibody titers at the time of transplantation and risk of disease recurrence.[23,59] The third suggests titers above 29RU/mL are highly sensitive and specific predictive markers of disease relapse.[60] *Seitz-Polski et al.* suggest however that persistently positive anti-PLA2R activity six months after kidney transplant is significantly associated with MN recurrence.[59] As such, PLA2R antibody testing should be considered prior to kidney transplantation in patients with history of PMN. Patients with significant PLA2R titers should be monitored closely post-transplant for proteinuria and graft disfunction. Repeated testing should be considered to document persistent PLA2R activity, when possible. Both *Seitz-Polski* and *Gupta et al.* suggest the use of stronger immunosuppressive regimens might constitute a protective factor for disease recurrence.[59,60] Preemptive rituximab therapy is another potential strategy.[60] Prospective studies with larger samples are needed to validate these findings and confirm the legitimacy of these approaches.

PLA2R studies are also of dubious value in children. Again, only three clinical trials regarding this population were included in this review. All showed serum anti-PLA2R antibody and renal PLA2R antigen positivity to be less frequent than in adult PMN patients, which suggests a different underlying etiology. Further studies are required in this area, preferably with greater and more ethnically diverse samples to avoid potential confounding factors.[61-63]

The identification of PLA2R's immunodominant epitope (a protein complex consisting of cysteine-rich, fibronectin-like type II, and C-type lectin-like domains) was an important breakthrough in this biomarker's relatively short lifespan.[66] *Seitz-Polski et al.* state that epitope spreading at baseline is an independent risk factor for non-remission and should be included in the therapeutic decision, depending on further confirmatory studies.[67]

To conclude, despite KB still being considered the gold standard for MN diagnosis, a noninvasive approach through anti-PLA2R antibody quantification is an increasingly pertinent alternative. Considering the high positive predictive value of anti-PLA2R testing for tissue diagnosis of PMN, KB may soon be considered a superfluous and invasive procedure in patients with stable renal function.[15] Sidelining KB in the diagnostic approach could imply a revision of the nomenclature associated with this disease, as a membranous histopathologic presentation would no longer be the hallmark feature of MN. However, most authors support a continued role for KB in patients presenting with severe azotemia, in spite of PLA2R positivity. Figure 2 shows a potential algorithm for noninvasive MN diagnosis applying current knowledge on PLA2R-associated biomarkers.

■ CONCLUSION

Anti-PLA2R antibody positivity in the context of nephrotic syndrome is very suggestive of MN. Despite occurring more frequently in primary

Figure 2

Proposed non-invasive diagnostic algorithm, based on *Bobart et al.*
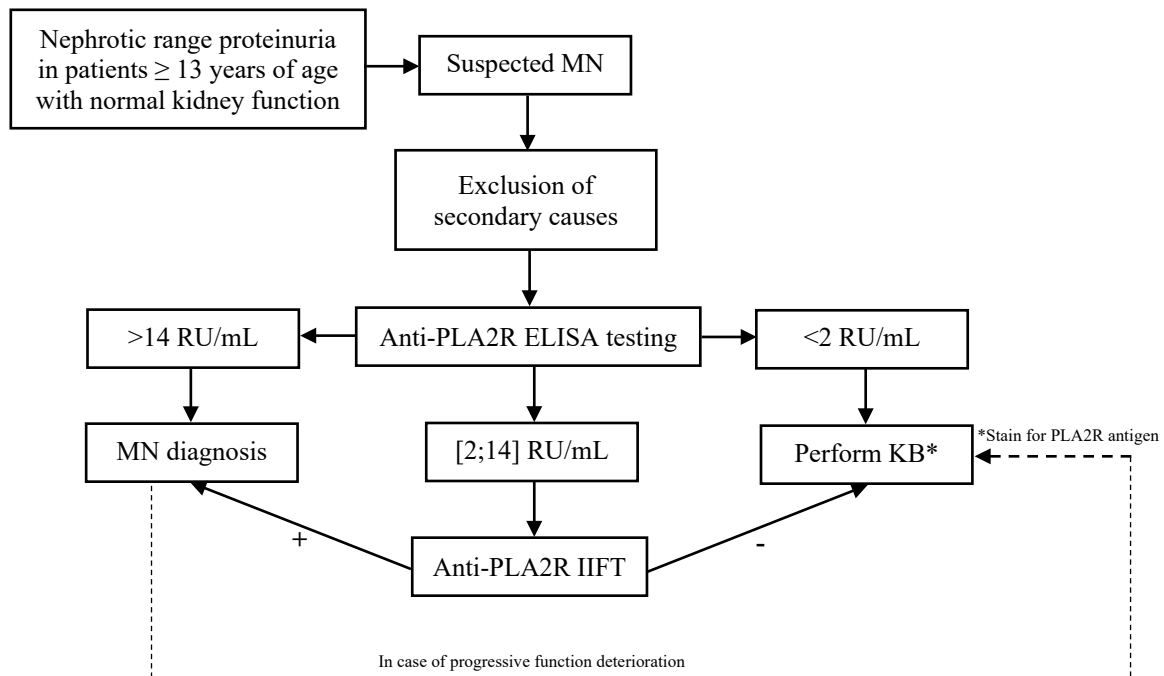
forms, exclusion of neoplasia and autoimmune disorders is still indispensable, as anti-PLA2R may also be present in secondary cases.

This biomarker is assuming an increasingly important role in the assessment of PMN activity, risk of recurrence and therapeutic monitoring. However, its interpretation must always be integrated with other markers of renal function. Immunoglobulin subtype characterization and PLA2R antigen staining can be useful to further characterize MN patients. IgG4 predominance supports PMN diagnosis, while dual positivity for PLA2R antigen deposits and anti-PLA2R antibodies might indicate increased disease severity and serve as supportive evidence for more aggressive therapeutic measures.

In the future, to increase this marker's clinical applicability, standardized cut-off values for seropositivity and for risk stratification must be set, taking into account the different detection methods used.

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