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# PLA2R Antibody Does Not Outperform Conventional Clinical Markers in Predicting Outcomes in Membranous Nephropathy

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**Introduction:** The prognostic value of PLA2R antibody (Ab) test in clinical practice remains unclear. We aimed to evaluate its ability in predicting hard outcomes in primary membranous nephropathy (PMN) after adjustments to conventional markers of disease activity.

**Methods:** A total of 222 patients diagnosed with PMN from January 2003 to July 2019 having had a serum PLA2R Ab test, were included from 3 centers in the north of England. Baseline conventional markers, PLA2R-Ab-status (positive vs. negative), Ab-titer (high vs. low), and time of testing (pre-PLA2R era vs. PLA2R era) were evaluated for association with outcomes. Primary outcome was time to progression (composite of doubling of creatinine, stage 5 chronic kidney disease, or death). Secondary outcomes were time to partial remission (PR) and time to immunosuppression. Cox proportional hazard testing was used.

**Results:** During a median follow-up of 5.26 years, progression was seen in 65 (29.3%) and PR in 179 of 222 patients (80.6%). There was a clear association of estimated glomerular filtration rate (eGFR) (standardized hazard ratio [$HR_Z$] = 0.767, $P < 0.05$) and urine protein-to-creatinine ratio (uPCR) ($HR_Z = 1.44$, $P < 0.005$) with time to progression among all patients, and eGFR ($HR_Z = 0.606$, $P < 0.005$) in Ab-positive patients. Baseline Ab-positivity was not associated with time to progression (adjusted hazard ratio [aHR] = 0.93, $P = 0.71$) or time to PR (aHR = 0.84, $P = 0.13$). Similarly, baseline high Ab-titer was not associated with time to progression (aHR = 1.07, $P = 0.77$) or time to PR (aHR = 0.794, $P = 0.08$).

**Conclusion:** Once adjusted to conventional markers of disease activity, baseline PLA2R Ab-positivity or Ab-titer do not predict disease progression or time to PR. Further studies are needed to harness the utility of PLA2R Ab test in prognostication in PMN.

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Discovery of the PLA2R antigen and autoantibody (PLA2R Ab) in 2009 was a landmark discovery in our understanding of PMN.[1] PMN is associated with varied prognoses ranging from spontaneous remission in about a third of patients to progressive renal dysfunction culminating into end-stage kidney disease in a 25% to 40% over a 10- to 15-year period. Before the discovery of PLA2R Ab in PMN, clinical management was guided by conventional biochemical markers, including proteinuria (measured as a uPCR or 24-hour protein), serum albumin, and kidney excretory function (measured as eGFR or creatinine clearance). These parameters define the activity of the disease and are easy to measure in routine clinical practice. A calculated approach with the use of immunosuppression is used in clinical practice to help avoid immunosuppression in patients who may achieve spontaneous remission. However, over the last 3 decades, such an approach based on conventional markers still led to significant proportion of patients progressing to end-stage kidney disease.[2-4] Since the emergence of PLA2R Ab testing, it has proven to be highly specific in diagnosing PMN and

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several studies have shown a strong association of Ab levels with proteinuria during period of 'watchful wait' and with treatment.[5-9]

There has been increasing interest in using this biomarker to stratify the likelihood of remission and risk of disease progression. A systematic review suggested that patients who are PLA2R Ab-negative are more likely to undergo spontaneous remission though the results from individual studies were conflicting.[10-13] Utility of PLA2R Ab in predicting clinical outcomes in comparison to the conventional clinical biomarkers was scrutinized in a few studies with variable findings. An updated meta-analysis of 11 studies with 824 patients found Ab positivity and high titers were associated with reduced clinical remission. However, the studies included in the meta-analyses were disparate and the association was evaluated primarily with the Ab-status (Ab-positive vs. Ab-negative) without adjustment for clinical phenotypic variables.[14] Jurubiță *et al.*[15] found that uPCR was a more important predictor of clinical outcomes compared with baseline PLA2R Ab status. To test the prognostic value of the anti-PLA2R-Ab titer, de Logt, *et al.*[16] used 2 Cox proportional hazard models using uPCR and serum creatinine with and without the PLA2R Ab test result in 168 patients. They observed that the model did not improve with the inclusion of PLA2R Ab-status or Ab-titer. Addition of data from 26 patients in the control arm from the GERMITUX group did not alter the results.[17]

Although the knowledge of PLA2R Ab test helps anticipate a treatment response it will not inform us about how the knowledge of the PLA2R Ab test at baseline can predict prognosis. Neither does it inform us if the test result can add further value beyond the conventional disease markers, proteinuria and renal excretory function, in predicting longer-term outcomes. Several outcomes are of interest in clinical practice, including patient and renal survival, PR, and decisions around immunosuppression treatment.

We aimed to study the effect of the PLA2R Ab test result and Ab-titer on disease outcomes focusing on the key renal outcomes of disease progression and remission. The objectives were to assess the association of both the PLA2R-Ab test status (positive vs. negative) and titers (high vs. low) with progression, PR, as well as time to immunosuppression, and to benchmark these parameters against conventional clinical markers. We also aimed to assess the association between the introduction of PLA2R Ab testing and changes in clinical outcomes since its discovery.

## METHODS

This was a retrospective longitudinal cohort study from 3 large specialist renal centers in the north of England (Manchester, Liverpool, and Sheffield). The cohort comprised 222 consecutive patients presenting with new incident nephrotic syndrome from January 2003 to July 2019, with follow-up data recorded through to September 2021. Patients were included if they had a diagnosis of PMN (biopsy-proven and/or clinical diagnosis with positive serum PLA2R Ab test). Baseline diagnosis of PMN was made by nephrologists after appropriate history, examination, autoimmune serology, malignancy, or systemic diseases were excluded. Among the total cohort, 10 patients were labeled as having PMN diagnosis without a renal biopsy. All those 10 patients had positive EUROIMMUN enzyme-linked immunosorbent assay (ELISA) PLA2R Ab test, eGFR >60 ml/min per 1.73 $m^2$, and had no other features to point to an alternative diagnosis for nephrotic syndrome other than membranous nephropathy (MN). We have excluded patients with subnephrotic proteinuria and an eGFR <60 ml/min per 1.73 $m^2$ to avoid other over-riding pathologies alongside MN, which may confound progression of kidney impairment (Supplementary Table S1). The study size was pragmatic based on all eligible patients.

Baseline characteristics included demographic details at diagnosis, markers of disease with eGFR and uPCR at presentation and PLA2R Ab test (positive vs. negative), Ab-titer (high vs. low), era (prediscovery vs. postdiscovery), and time of test (contemporary vs. retrospective).

Baseline values were defined as the laboratory values closest to the date of diagnosis. We defined PLA2R Ab-positivity as a positive result from either an ELISA using Manchester in-house or EUROIMMUN testing or indirect immunofluorescence (IF) assay. From 2016 onwards, all PLA2R-Ab tests for ELISA were performed by the Sheffield Protein Reference Unit using the EUROIMMUN kit with levels <14 RU/ml interpreted as negative, 14 to 20 RU/ml as borderline, and >20 RU/ml as positive. For anti-PLA2R Ab ELISA tests performed using the in-house Manchester ELISA, cut off positivity of ≥40 RU/ml was used as previously reported.[18] Indirect IF testing was performed with EUROIMMUN kit being the most commonly available commercial test as previously reported.[19] The number of patients who were tested by either of the 3 methods were $n = 110$, $n = 76$, and $n = 36$ using in-house Manchester ELISA kit, EUROIMMUN kit, and IF assay, respectively (Supplementary Table S2).

Cutoff values for PLA2R Ab-titers have not been validated previously.[5] We defined "high titers" as ≥150 RU/ml on ELISA or ≥1/100 on IF assay in those testing positive and "low titers" as <150 RU/ml

on ELISA and <1/100 on IF assay.[20] The prediscovery era was defined as those who had a biopsy and blood tests performed before January 1, 2012, and the post-discovery era was after this time. The "contemporary" group was defined as those who had their serum PLA2R Ab test performed within 6 months of biopsy during the postdiscovery era. Therefore, clinicians had access to bloods at time of diagnosis, which might alter their decision making and impact the outcomes. The aforementioned group was compared with a retrospective group, where clinicians had no access to bloods at time of diagnosis. Before 2012, patients recruited in MN studies in Manchester (under ethics references 06/Q1401/5 and 10/H1008/10) had their stored samples analyzed in retrospect for PLA2R Ab by ELISA. These patients and those presenting after January 1, 2012 without a test within 6 months of presentation formed the "retrospective" group (Supplementary Figure S1).

The primary end point was time to disease progression. "Progression" was defined as a composite outcome of doubling of serum creatinine, stage 5 chronic kidney disease, or death (whichever is earliest). To avoid the bias of older age in our cohort that might be a reason for death by its own, we have repeated our analysis focusing on hard renal end points (Supplementary Tables S3–S5).

Secondary end points were PR (defined as uPCR <350 mg/mmol and reduction of >50% compared with baseline), and first immunosuppression. We analyzed the following:

- association of serum PLA2R Ab status (positive vs. negative) with outcomes
- association of Ab titers (for PLA2R Ab-positive patients – high vs. low titer) with outcomes
- association of PLA2R Ab-titers on initiation of immunosuppression
- association of the timing of the test (contemporaneously or retrospectively) on outcomes

Analyses were done by grouping data into 2 analysis sets as follows:

- The full analysis set: all patients with PLA2R Ab (to compare Ab-positive with Ab-negative groups)
- The titer analysis set: those with a positive PLA2R Ab test (to compare high with low Ab-titer groups).

Analyses for time to immunosuppression were done excluding any patients with prior immunosuppression use. Immunosuppression included steroids, calcineurin inhibitors, alkylating agents, mycophenolate mofetil, and rituximab.

We applied survival methodology for data analyses. "Time-zero" was the date of the biopsy or PLA2R test

**Table 1.** Clinical characteristics in anti-PLA2R Ab positive, negative, high titer, and low tire patients with PMN

| Variables | PLA2R-Ab Positive $n = 151$ | PLA2R-Ab Negative $n = 71$ | PLA2R-Ab Positive | |
|---|---|---|---|---|
| | | | High Titer $n = 81$ | Low Titer $n = 70$ |
| Age at diagnosis (years) | | | | |
| Mean (SD) | 59.3 (13.58) | 60.3 (15.13) | 58.3 (13.59) | 60.5 (13.57) |
| Sex | | | | |
| Male | 116 (76.8%) | 38 (53.5%) | 65 (80.2%) | 51 (72.9%) |
| Female | 35 (23.2%) | 33 (46.5%) | 16 (19.8%) | 19 (27.1%) |
| Ethnicity | | | | |
| White | 125 (82.8%) | 54 (76.1%) | 60 (74.1%) | 65 (92.9%) |
| Asian | 13 (8.6%) | 6 (8.5%) | 10 (12.3%) | 3 (4.3%) |
| Black | 7 (4.6%) | 2 (2.8%) | 7 (8.6%) | 0 |
| Mixed | 1 (0.7%) | 0 | 1 (1.2%) | 0 |
| Not stated | 5 (3.3%) | 9 (12.7%) | 3 (3.7%) | 2 (2.9%) |
| Time in follow-up (years) | | | | |
| Median (IQR) | 5.3 (2.5–9.0) | 3.9 (2.2–6.3) | 4.0 (2.2–6.8) | 7.5 (3.4–10.2) |
| Total follow-up (patient-years) | 914.4 | 339.1 | 395.7 | 518.7 |
| Era | | | | |
| Prediscovery | 54 (35.8%) | 14 (19.7%) | 15 (18.5%) | 39 (55.7%) |
| Postdiscovery | 97 (64.2%) | 57 (80.3%) | 66 (81.5%) | 31 (44.3%) |
| Time of PLA2R-Ab sample | | | | |
| Contemporary | 82 (54.3%) | 39 (54.9%) | 61 (75.3%) | 21 (30.0%) |
| Retrospective | 69 (45.7%) | 32 (45.1%) | 20 (24.7%) | 49 (70.0%) |
| eGFR (ml/min per 1.73 $m^2$) | | | | |
| Mean (SD) | 68.1 (25.78) | 67.2 (28.48) | 63.0 (26.51) | 73.9 (23.77) |
| uPCR (mg/mmol) | | | | |
| Mean (SD) | 655.3 (322.95) | 740.9 (439.41) | 721.1 (333.61) | 579.1 (294.48) |

Ab, antibody; eGFR measured in ml/min per 1.73 $m^2$; IQR, interquartile range; PMN, primary membranous nephropathy; uPCR measured in mg/mmol.

(if kidney biopsy was not performed). Censoring was at last follow-up or death. We used Cox proportional hazards testing to evaluate the association of the variable in question with outcomes. Adjusted hazard ratio, aHR, is the hazard ratio (HR) after controlling for eGFR and uPCR and is presented with 95% confidence intervals. HR in the tables is presented along with the $HR_Z$, denoting HR when the covariate has been standardized. HR is presented as the risk relative to the following reference groups: negative PLA2R Ab, low Ab-titer; retrospective test during the prediscovery era. Complete-case analysis was used; however, there was very little missing data. Subgroups by partition were not analyzed. There were no sensitivity analyses. We censored the use of immunosuppression in 1 model to elucidate the natural course of the disease. Survival analysis was also presented using Kaplan–Meier plots.

Analysis was performed using R v4.0.5 (R Core Team, Vienna, Austria). $P < 0.05$ was considered significant. The study is reported in line with STROBE guidelines.

## RESULTS

A total of 340 patients were initially recorded in the data set. Of these, 2 patients were excluded because of either being in stage 5 chronic kidney disease or with a combination of eGFR <60 and uPCR <350 at presentation. In addition, 89 were excluded for lack of PLA2R Ab test. Two patients were excluded having had previous history of nephrotic syndrome. A total of 222 patients were included in the primary analysis set, 151 in the Ab-positive group and 71 in Ab-negative group. Of the patients, 81 and 70 had "high" and "low" Ab titers, respectively. Median follow-up was 5.26 years.

### Baseline Characteristics

Baseline characteristics are outlined in Table 1. Age and eGFR were similar in all the groups; there was a higher proportion of men in the Ab-positive group ($P < 0.001$). Although there appeared to be higher baseline uPCR in the PLA2R Ab-negative group (740.9 mg/mmol vs. 655.3 mg/mmol), this was not statistically significant. There was a significant difference in both eGFR and uPCR between high and low-titer groups (Figure 1); low Ab-titers were associated with lower uPCR ($P = 0.006$) and higher eGFRs ($P = 0.008$; Figure 1).

### Outcomes

Outcomes in different groups, number of events, and event-free patient-years during follow-up are presented in Table 2.

### Anti-PLA2R-Ab-Positive Versus Negative Groups

There was no observed difference in time to progression between PLA2R Ab-positive and Ab-negative groups (aHR = 0.933 [0.645–1.348], $P = 0.710$; Table 3 and Figure 2). However, eGFR and uPCR in the Ab-status model were significantly associated with disease progression (Table 3). There was no significant difference in time to PR between PLA2R Ab-positive and Ab-negative group (aHR = 0.843 [0.675–1.053], $P = 0.132$; Table 4). Time to immunosuppression was significantly shorter in the PLA2R Ab-positive group compared with Ab-negative group (aHR = 1.450 [1.104–1.905], $P = 0.008$; Table 5), eGFR and uPCR (Table 5).

### PLA2R Ab-High Versus Ab-Low Groups

The high-titer group was not associated with time to progression (aHR = 1.069 [0.676–1.690], $P = 0.775$). The eGFR in the titer model was significantly associated with disease progression (HRz = 0.606 [0.430–0.854], $P = 0.004$; Table 6). There seemed to be a trend, although not statistically significant, toward shorter time to PR in the low-titer group compared with the

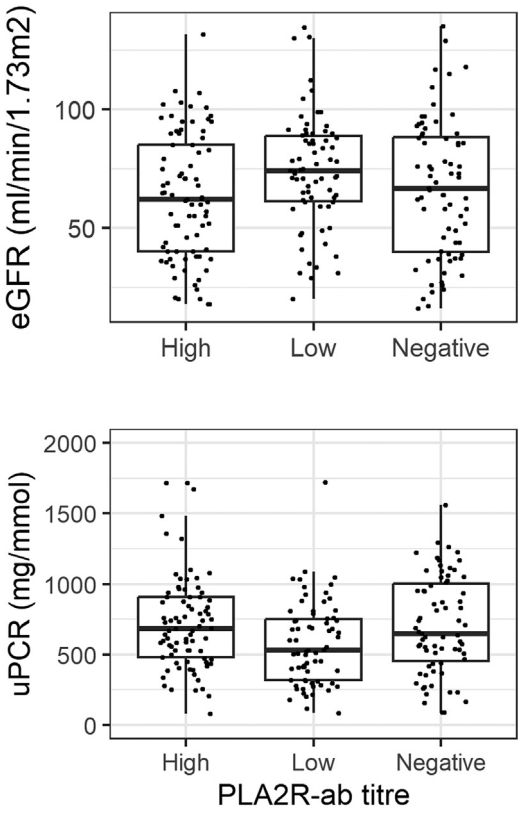


**Figure 1.** Modified side by side plots correlating different PLA2R Ab levels to eGFR and uPCR. Ab, antibody; eGFR measured in ml/min per 1.73 $m^2$; uPCR measured in mg/mmol.

**Table 2.** Number of events and event-free per year in anti-PLA2R Ab positive, negative, high titer, and low tire patients with PMN

| Variables | PLA2R-Ab positive $n = 151$ | PLA2R-Ab negative $n = 71$ | PLA2R-Ab positive | |
|---|---|---|---|---|
| | | | High titer $n = 81$ | Low titer $n = 70$ |
| Progression | 43/151 (28.5%) | 22/71 (31.0%) | 24/81 (29.6%) | 19/70 (27.1%) |
| Median (IQR) event-free years | 4.1 (1.7–8.1) | 3.7 (1.6–5.3) | 3.3 (1.5–6.5) | 6.7 (3.0–9.9) |
| Death | 21/151 (13.9%) | 12/71 (16.9%) | 9/81 (11.1%) | 12/70 (17.1%) |
| Median (IQR) event-free years | 5.5 (2.5–9.0) | 3.9 (2.2–6.6) | 3.9 (2.2–6.8) | 7.5 (3.4–10.2) |
| Partial remission | 121/151 (80.1%) | 58/71 (81.7%) | 62/81 (76.5%) | 59/70 (84.3%) |
| Median (IQR) event-free years | 1.1 (0.7–2.0) | 0.8 (0.4–1.5) | 1.3 (0.8–2.4) | 1.0 (0.6–1.6) |
| Immunosuppression | 103/146 (70.5%) | 35/70 (50.0%) | 66/79 (83.5%) | 37/67 (55.2%) |
| Median (IQR) event-free years | 0.6 (0.2–3.1) | 1.0 (0.3–3.8) | 0.5 (0.3–1.5) | 1.2 (0.3–5.5) |

Ab, antibody; IQR, interquartile range; PMN, primary membranous nephropathy.

high-titer group (aHR = 0.794 [0.612–1.029], $P$ = 0.081; Table 7 and Figure 3).

## PLA2R Ab Test on Immunosuppression Initiation

Patients with high Ab-titer had significantly shorter time to starting immunosuppression than those with low Ab-titers (aHR = 1.421 [1.056–1.912], $P$ = 0.020; Table 8 and Figure 4). Contemporary knowledge of PLA2R Ab-positivity and high Ab-titers were associated with shortened time to immunosuppression (Supplementary Tables S6 and S7).

## Anti-PLA2R-Ab Test Contemporary Versus Retrospectively

We further evaluated the relationship between PLA2R Ab assay analyzed at different times with presentation (contemporary vs. retrospective) and times to progression and PR. With time to progression, there was a lack of association with the Ab-titer, the time of the assay, or the interaction between the 2 (Supplementary Table S8). However, there was a significant effect of both Ab-titer and timing of the test with time to PR, although there was no significant interaction between the 2 (Supplementary Table S9).

To describe the natural history of the disease, we analyzed the outcomes (time to progression and time to PR) with Ab-titers after censoring for use of immunosuppression. For time to progression, there was no significant association with high Ab-titer (aHR = 1.198 [0.581–2.470], $P$ = 0.624) but significantly shorter time to PR than with low Ab-titers (aHR = 0.517 [0.321–0.833], $P$ = 0.007). It was noted that eGFR was strongly associated with time to disease progression but not time to PR (Supplementary Table S10 and S11).

**Table 3.** Cox PH model for Time to Progression by anti-PLA2R-Ab status adjusted for eGFR and uPCR

| Variables | HR | $HR_Z$ | $P$ |
|---|---|---|---|
| PLA2R Ab-positive | 0.933 (0.645–1.348) | | 0.710 |
| eGFR | 0.990 (0.980–1.000) | 0.767 (0.591–0.996) | 0.047 |
| uPCR | 1.001 (1.000–1.002) | 1.440 (1.124–1.845) | 0.004 |

Ab, antibody; HR, hazard ratio; $HR_Z$, standardized hazard ratio; eGFR measured in ml/min per 1.73 $m^2$, uPCR measured in mg/mmol.
$P < 0.05$ was considered significant.

# DISCUSSION

Despite being more than a decade since the discovery of the PLA2R Ab and testing in clinical practice, its utility as a prognostic marker is yet to be fully explored. Most of the earlier studies evaluated the association of PLA2R Ab results with the clinical phenotype (proteinuria) and treatment response (using rituximab).[8,9,17] Evidence thus far shows a strong correlation of Ab titers with proteinuria and that immunologic remission following immunosuppression precedes clinical remission.

In this large, tri-center, retrospective longitudinal cohort study, we find that conventional biomarkers outperformed both PLA2R Ab-status (positive vs. negative) and Ab-titer (high vs. low Ab-titers) in predicting time to disease progression. There was no significant difference in baseline proteinuria and renal excretory function between Ab-positive and Ab-negative groups. Although time to immunosuppression was shorter in Ab positive group, this has not delayed the disease progression when adjusted to baseline proteinuria and eGFR. eGFR and proteinuria remained key determinants of disease progression, measured as a composite of doubling of serum creatinine, renal and patient survival.

Baseline proteinuria was lower and eGFR was higher in low Ab-titer group compared with high Ab-titer group. Proportion and time to immunosuppression were significantly longer in low Ab-titer group. However, when adjusted to baseline uPCR and eGFR, there was no significant difference in time to progression or time to PR between the high and low Ab-titer groups. Knowledge of the anti-PLA2R-Ab status/titer may have influenced the decision to start immunosuppression; however, this has not reflected on longer-term hard renal outcomes.

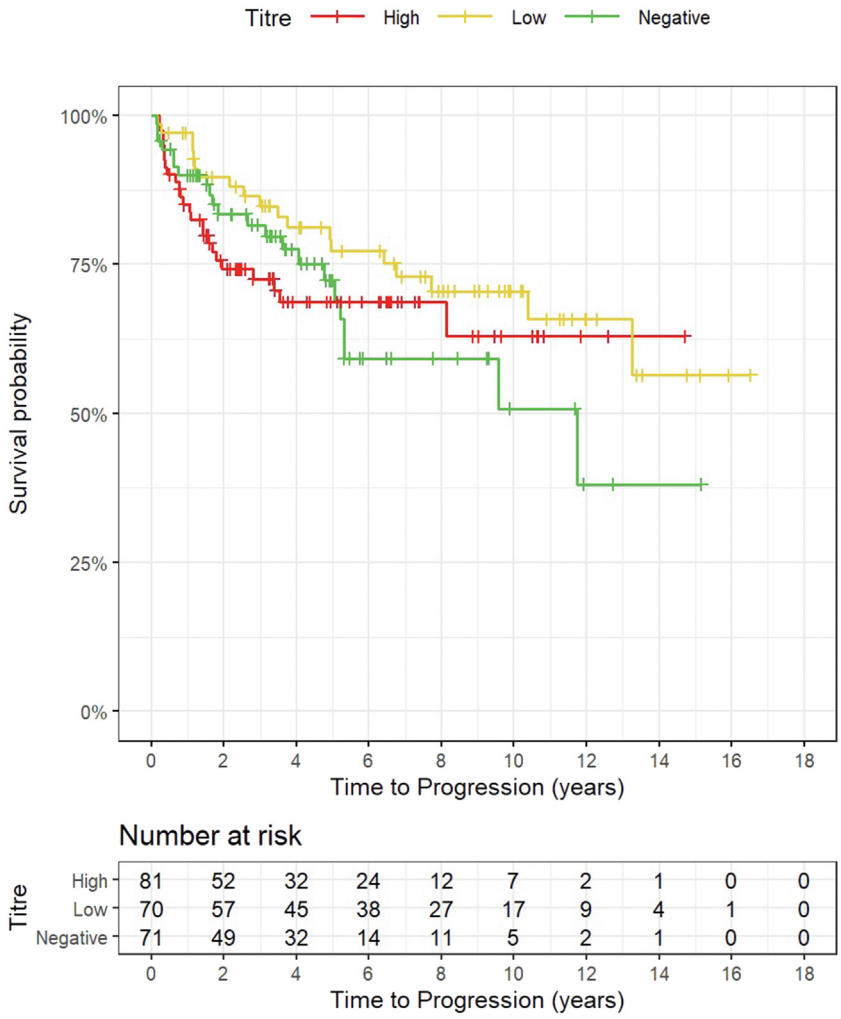


**Figure 2.** Time to progression by PLA2R Ab test status and Ab-titer. Ab, antibody.

These results are inconsistent with the recent meta-analysis, which mainly focused on spontaneous remission as an end point.[13] Given the small number of studies, results from this meta-analysis may need interpretation with caution because the power of meta-analysis was only 56.6% and did not focus on time to disease progression as an end point. It is possible that the lack of adjustment to baseline variables including eGFR and uPCR in previous studies may exaggerate the apparent effect of Ab burden on outcomes.

We also noticed that conventional disease markers in the PLA2R Ab-status model, were very strong predictors of time to disease progression, although a similar association was not found with time to PR. This can be explained by the known lag effect of clinical remission behind immunologic remission, and we speculate that the use of immunosuppression may influence PR much further than their effect on disease progression.

In contrast to our finding that PLA2R Ab-status has no significant effect on disease progression or remission after adjusting for baseline eGFR and uPCR, Qin *et al.*[21] found that patients with PLA2R Ab-negative status experienced a higher remission rate and slower disease

**Table 4.** Cox PH model for time to partial remission by anti-PLA2R-Ab status adjusted for eGFR and uPCR

| Variables | HR | $HR_Z$ | *P* |
|---|---|---|---|
| PLA2R Ab-positive | 0.843 (0.675–1.053) | | 0.132 |
| eGFR | 1.000 (0.994–1.006) | 1.007 (0.857–1.184) | 0.930 |
| uPCR | 1.000 (0.999–1.000) | 0.930 (0.785–1.102) | 0.403 |

Ab, antibody; HR, hazard ratio; HRZ, standardized hazard ratio; eGFR measured in ml/min per 1.73 m2; uPCR measured in mg/mmol.
$P < 0.05$ was considered significant.

**Table 5.** Cox PH model for Time to initiate immunosuppression by anti-PLA2R-Ab status adjusted for eGFR and uPCR

| Variables | HR | $HR_Z$ | *P* |
|---|---|---|---|
| PLA2R Ab-positive | 1.450 (1.104–1.905) | | 0.008 |
| eGFR | 0.991 (0.984–0.997) | 0.778 (0.649–0.933) | 0.007 |
| uPCR | 1.001 (1.000–1.001) | 1.296 (1.113–1.510) | <0.001 |

Ab, antibody; HR, hazard ratio; HRZ, standardized hazard ratio; eGFR measured in ml/min per 1.73 m2; uPCR measured in mg/mmol.
$P < 0.05$ was considered significant.

**Table 6.** Cox PH model for Time to Progression by anti-PLA2R-Ab titer adjusted for eGFR and uPCR

| Variables | HR | $HR_Z$ | *P* |
|---|---|---|---|
| High Ab-titer | 1.069 (0.676–1.690) | | 0.775 |
| eGFR | 0.981 (0.968–0.994) | 0.606 (0.430–0.854) | 0.004 |
| uPCR | 1.001 (1.000–1.001) | 1.224 (0.885–1.694) | 0.223 |

Ab, antibody; HR, hazard ratio; HRZ, standardized hazard ratio; eGFR measured in ml/min per 1.73 m2; uPCR measured in mg/mmol.
$P < 0.05$ was considered significant.

progression rate than those with Ab-positive status. Although this may well be true, the effect may have been blunted by the difference in baseline eGFR between both groups, which was significantly different. Jurubiță *et al.*[15] reached the same conclusion as Qin *et al.*[21] that the PLA2R Ab-negative group carries a better clinical outcome compared with the PLA2R Ab-positive group. Again, this effect can be attributed to the lower uPCR and higher eGFR identified in the negative status group rather than the Ab-status *per se*.

There was no association between PLA2R Ab-titers and the time to progression and limited impact on time to PR in favor of the low-titer group. We notice a strong relationship between eGFR and time to progression. eGFR is highly correlated with progression, and it has far more effect than titer, *per se*, even though there may be a causal link between titer and eGFR (Figure 1). We speculate that this association of high Ab-titer with eGFR may blunt its effect with prediction of progression. There may be other factors that affect eGFR at presentation and outcomes that were not observed here. And it is not surprising that eGFR has a lot more significant effect on disease progression, which is an outcome defined by a decline in eGFR itself. Our findings bring to question the direct relevance of the baseline PLA2R Ab test as a prognostic marker, as was noted in the recent study by de Logt *et al.*[16]

The recently published Kidney Disease: Improving Global Outcomes guidelines recommends use of immunosuppression treatment not only for patients at risk of renal disease progression, but also for those suffering from severe/life-threatening nephrotic syndrome irrespective of PLA2R Ab test result.[5] Therefore, progressive renal dysfunction and/or severe life-threatening nephrotic syndrome should be considered as indications for immunosuppression treatment. We find that the "contemporary" assay and its result may have affected clinical decision with a significant trend toward earlier immunosuppression in the Ab-positive as well as the high Ab-titer groups. After censoring for the use of immunosuppression, however, no difference in time to progression was observed between high versus low Ab-titer groups. However, there were only 16 patients who reached the end point of progression before initiation of immunosuppressants. Studying immunosuppressant-naïve patients to see the natural course of the disease was limited because many patients received immunosuppression shortly after presentation and before any events occurred, leading them to be censored in this particular analysis.

**Table 7.** Cox PH model for time to partial remission by anti-PLA2R-Ab titer adjusted for eGFR and uPCR

| Variables | HR | $HR_Z$ | *P* |
|---|---|---|---|
| High Ab-titer | 0.794 (0.612–1.029) | | 0.081 |
| eGFR | 0.997 (0.990–1.005) | 0.935 (0.762–1.149) | 0.524 |
| uPCR | 1.000 (0.999–1.000) | 0.924 (0.747–1.142) | 0.462 |

Ab, antibody; HR, hazard ratio; HRZ, standardized hazard ratio; eGFR measured in ml/min per 1.73 m2; uPCR measured in mg/mmol.
$P < 0.05$ was considered significant.

One needs to appreciate that a negative PLA2R Ab test could be due to another target glomerular antigen.[22] Studies have established that 70% to 80% of PMN cases possess circulating autoantibodies targeting the M-type PLA2R on the podocytes. Several other antigen-Ab phenomena have been discovered in recent times. Therefore, PMN with a negative PLA2R Ab test because of a different target antigen might result in a different clinical phenotype (either less or more severe) and this requires further larger multicenter studies, with each Ab being associated with PMN in only a minority of patients.

Glomerular staining can be considered an additional tool to serum antibodies in the diagnostic toolkit for MN. However, it is not always available and the few studies investigating prognostic value failed to show consistent results with glomerular staining. Liu *et al.*[10] found a more beneficial response to immunosuppression in patients with PLA2R expression. However, the analysis did not include an adjustment for baseline renal parameters.[10] The sample size was small in the study of Wang *et al.*,[11] particularly in the non-PLA2R-associated PMN group. Zhang *et al.*[12] did not adjust for baseline proteinuria, which was significantly higher in the PLA2R-associated PMN group.

The main power of our study is contributed by the addition of the historical group with Ab testing in retrospect, especially before 2012 when routine testing was unavailable in clinical practice. Equally important was the multicenter nature of our study across the same period, and adjustment of Ab test results with baseline renal clinical parameters. These factors provide further insights into changes in clinical practice and their potential influence on clinical outcomes.

Our findingshave limitations. We acknowledge the study's retrospective nature; however, studying long-term outcomes prospectively in rare diseases, like MN, is enormously challenging especially with outcomes occurring after several months or years. Although there

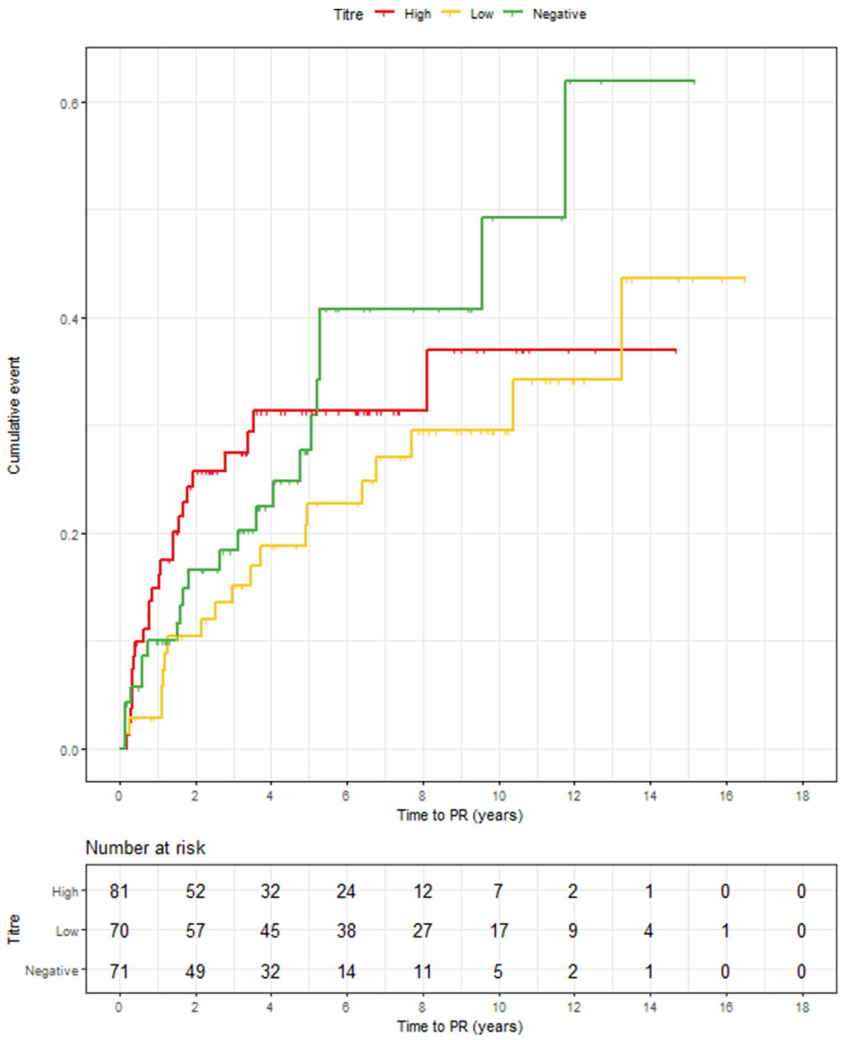


**Figure 3.** Time to partial remission by PLA2R Ab test status and Ab-titer. Ab, antibody.

are benefits of including multiple centers in the study, for example, greater generalizability, different methods of testing (ELISA using in-house/EUROIMMUN kits and IF assays) have meant that we have had to attempt to categorize titers measured on2 different scales. Also, we arbitrarily divided the cohort to high versus low Ab levels of $>$ or $<$ 150 RU/ml. This will need validation in future studies. For some end points, this study has a smaller number of events than would have been desired and led to underpowered comparisons, so even though the HRs calculated appear substantial, they do not reach statistical significance. There were imbalances in some subgroups which occurred naturally because of the retrospective nature of the study. It is possible that serial PLA2R Ab testing may lend additional benefit in predicting longer-term prognosis but is outside the scope of this study.

**Table 8.** Cox PH model for Time to initiate immunosuppression by anti-PLA2R-Ab titer adjusted for eGFR and uPCR

| Variables | HR | $HR_Z$ | *P* |
|---|---|---|---|
| High Ab-titer | 1.421 (1.056–1.912) | | 0.020 |
| eGFR | 0.990 (0.982–0.999) | 0.769 (0.615–0.962) | 0.022 |
| uPCR | 1.001 (1.000–1.001) | 1.216 (0.978–1.512) | 0.079 |

Ab, antibody; HR, hazard ratio, HRZ, standardized hazard ratio; eGFR measured in ml/min per 1.73 m2; uPCR measured in mg/mmol.
$P < 0.05$ was considered significant.

In conclusions, the PLA2R Ab test has shown great promise as a diagnostic marker in MN, but test results should be interpreted cautiously when it comes to prognostication. Our findings describe how both PLA2R Ab-status (positive or negative) and Ab-titer (high or low) may not significantly affect the hard renal end point of disease progression. Although time to immunosuppression was shorter in the high-titer group, it is unclear if this has influenced PR. We demonstrate that conventional disease markers, like eGFR, remain key in predicting longer-term outcomes like progression, with Ab titers possibly helping to predict time to PR.

This study brings to fore the important question around the utility of the PLA2R Ab test result in

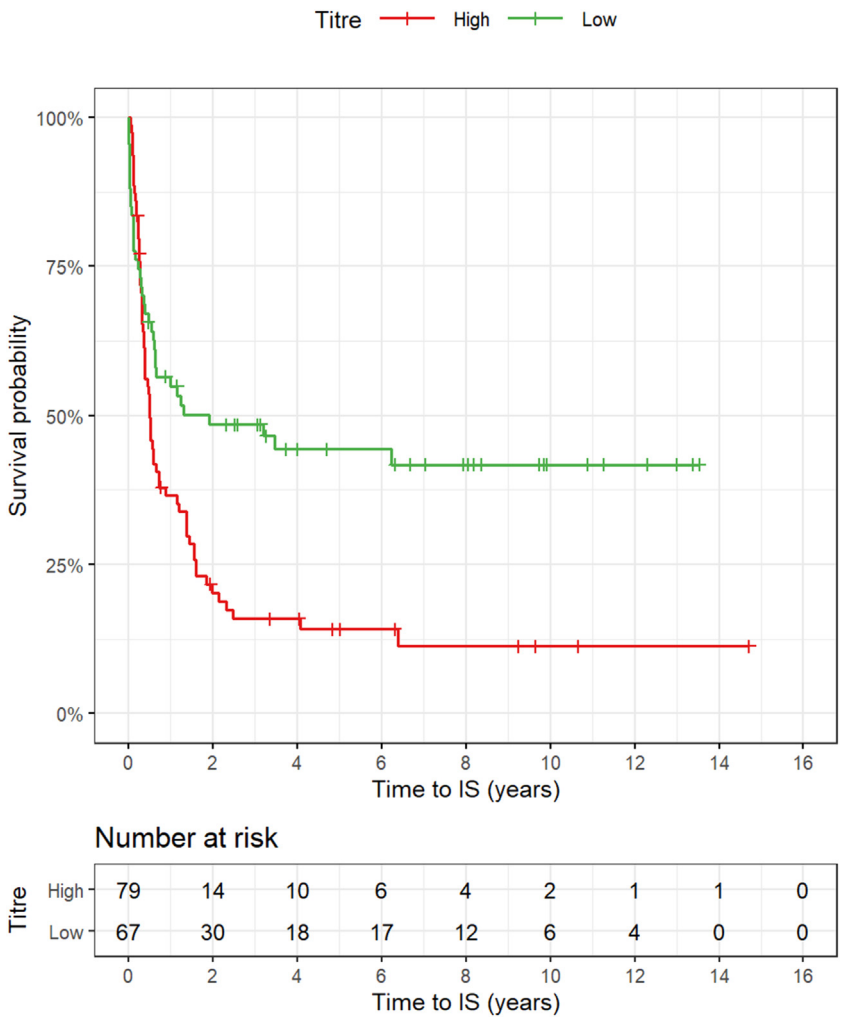


**Figure 4.** Time to immunosuppression treatment by PLA2R Ab-titer. *Ab, antibody.*

predicting prognosis in clinical practice. We urge that larger multiyear, multicenter studies are undertaken, through national and international registries as well as through serial Ab testing to help unravel their utility further. Until more evidence emerges, we support use of multiple risk factors to determine prognosis and treatment strategies in clinical practice.

## DISCLOSURE

All the authors declared no competing interests.

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## DATA AVAILABILITY STATEMENT

Data are available under reasonable request to the corresponding author.

## AUTHOR CONTRIBUTIONS

OR assisted in designing the study, performing the literature review, and drafting the manuscript; SBa assisted in designing the study, analyzing the data, and assisted in writing the manuscript; SBu, MH, SS, and AK assisted in data collection and reviewed the manuscript; AR conceptualized the study, assisted in designing the study, data collection and analyzing the data, and reviewed the manuscript; DAK conceptualized the study, assisted in designing the study, data collection, and assisted in writing the manuscript. OR is the author guarantor for responsible for the overall content.

## ETHICAL APPROVAL

Not specifically required for this study but data were reused from previous studies approved under ethics references 06/Q1401/5 and 10/H1008/10.

## SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

**Supplementary Table S1.** The proportion of patients included/excluded based on their eGFR and uPCR.

**Supplementary Table S2.** The proportion of patients who were tested by ELISA test (in-house/EUROIIMUN) and indirect immunofluorescence test.

**Supplementary Table S3.** Number of events and event-free per year in Anti-PLA2R Ab Positive, Negative, high titer, and low tire patients with PMN looking at hard renal outcome (DSC/CKD5).

**Supplementary Table S4.** Cox PH model for Time to Progression by Anti-PLA2R-Ab status adjusted for eGFR and uPCR (only looking into hard renal endpoints excluding death).

**Supplementary Table S5.** Cox PH model for Time to Progression by Anti-PLA2R-Ab titer adjusted for eGFR and uPCR (only looking into hard renal endpoints excluding death).

**Supplementary Table S6.** Cox proportional hazard model for immunosuppression by time of test and titer (with interaction) adjusted for eGFR and uPCR.

**Supplementary Table S7.** Cox proportional hazard model for time to immunosuppression by the timing of test and titer (with interaction) adjusted for eGFR and uPCR.

**Supplementary Table S8.** Cox proportional hazard model for Time to Progression by the timing of test and titer (with interaction) adjusted for eGFR and uPCR.

**Supplementary Table S9.** Cox proportional hazard model for Time to Partial Remission by the timing of test and titer (with interaction) adjusted for eGFR and uPCR.

**Supplementary Table S10.** Cox proportional hazard model for Time to Progression by titer adjusted for eGFR and uPCR and censored at immunosuppression.

**Supplementary Table S11.** Cox proportional hazard model for Time to Partial Remission by titer adjusted for eGFR and uPCR and censored at immunosuppression.

**Supplementary Figure S1.** Flow chart for the timing of PLA2R-Ab samples collected in our study (retrospectively/contemporary) determined by clinicians' access to results at time of diagnosis.

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