UNIVERSITY OF MEDICINE AND PHARMACY
„CAROL DAVILA", BUCHAREST

DOCTORAL SCHOOL

MEDICINE

THE ROLE OF ANTI-PHOSPHOLIPASE A2 ANTIBODY IN DIAGNOSTIC AND MANAGEMENT OF PRIMARY MEMBRANOUS NEPHROPATHY

PhD THESIS SUMMARY

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Table of content

Introduction

Primary membranous nephropathy (pMN) is an autoimmune glomerulopathy determined by autoantibodies targeting antigens expressed on the podocyte's surface(1**)**. Although the incidence has declined over the past decades, mostly related to the dramatic increase in the frequency of diabetic nephropathy, pMN remains an important cause of NS in nondiabetic adults, affecting mostly Caucasians with ages between 30 and 50 year(2).

Since the landmark study in 2009 describing the first human target autoantigen, M-type phospholipase A2 receptor 1 (PLA2R)(3) the landscape of pMN has significantly changed with the description of several new putative "antigens" (THSD7A, EXT1/EXT2, NELL1, Sema3B, PCDH7)(4,5). Accordingly, a formerly exclusive histological diagnosis has merely become a pattern of glomerular injury with a serology-driven etiological classification, as each distinct antigen is likely associated with a different pathophysiological phenomenon (4). However, anti-PLA2R antibodies remain the most important contributors to pMN etiology, accounting for up to 50-80% of cases(5).As murine PLA2R1 shares a moderate (≈70%) amino acid identity with human PLA2R1, an adequate experimental model to fully demonstrate the anti-PLA2R antibody pathogenicity is still lacking(6). Nevertheless, there is substantial clinical evidence to support their role in disease pathogenesis (1). In addition to their diagnostic role, anti-PLA2R strongly correlate with disease activity as they have been shown to appear months to years prior to clinical manifestations of pMN, disappear prior to clinical remission, reappear prior to a relapse and predict post-treatment outcome(1,7-12).

Because the prevalence of anti-PLA2R antibodies (anti-PLA2R ab) is high in nephrotic syndrome (NS) caused by pMN, in contrast to NS both to secondary MN (sMN) or other causes, a positive anti-PLA2R serology was proposed as a diagnostic tool intended to avoid kidney biopsy in patients with NS. Many studies investigated the utility of anti-PLA2R ab in differentiating pMN from sMN or other causes of nephrotic syndrome, using kidney biopsy as control.(1) However, as the number of patients was rather low and different methods of assessment were used – indirect immunofluorescence, ELISA, Western blot – a cut-off level was not yet defined(1).

A recent metanalysis investigated the utility of PLA2R serology to differentiate pMN from sMN or from non-MN in nephrotic patients, and concluded that the diagnostic accuracy was overall good(13). However, the heterogeneity of studies was high, and

subgroup analyses suggested that diagnostic accuracy depended on method of anti-PLA2R ab assessment and some patients' characteristics, i.e. the degree of proteinuria. More recently, Bobart *et al* suggested that among patients with preserved kidney function and no evidence of secondary causes, a positive anti-PLA2R serology highly predicts a diagnosis of pMN, supporting that anti-PLA2R ab diagnostic accuracy varies according to patients' characteristics (14).

Accordingly, we sought to investigate the diagnostic accuracy of anti-PLA2R antibodies for pMN in a cohort of consecutive patients with NS in which, as part of the initial work-up, anti-PLA2R antibodies were also screened.

Material and Methods Study 1

Study design and population

We conducted a cross-sectional study that included 203 consecutive patients diagnosed with nephrotic syndrome (NS) between January 2015 and December 2019 at our department. Nephrotic syndrome (NS) was defined as 24-h proteinuria over 3.5 g/day in association with hypoalbuminemia (serum albumin below 3/5 g/dl). All patients underwent a systematic screening for secondary causes of NS such as autoimmune disorders, hepatitis serology, age-appropriate malignancy screening, monoclonal gammapathy evaluation and medication history. Additionally, as part of the initial work-up of the NS all patients had anti-PLA2R antibody determination. Subsequently, all patients underwent a kidney biopsy to confirm the histological diagnosis, irrespective of the anti-PLA2R serology. Exclusion criteria were: age < 18 years-old, patients with positive PLA2R serology without a confirmatory kidney biopsy, patients with PLA2R determination after histological diagnosis of pMN, patients without NS, patients with prior immunosuppression.

The study was conducted with the provisions of the Declaration of Helsinki and the protocol was approved by the local ethics committee (The Ethics Council of Fundeni Clinical Institute, Registration number: 8851).

Data collection

Baseline data were collected via electronic medical record review of patients at the time of NS diagnosis and included: age at presentation, gender, comorbidities, PLA2R antibodies titer, serum creatinine, albumin and total proteins, uric acid, lipid panel, serum

fibrinogen, hemoglobin, 24-h proteinuria and hematuria. Glomerular filtration rate (eGFR) was estimated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.

Plasma samples for anti-PLA2R antibody determination were collected concomitantly with those for baseline laboratory data evaluation and those used for screening of potential secondary causes of NS, prior to kidney biopsy. Anti-PLA2R antibodies were determined by an ELISA assay (EUROIMMUN AG, Lübeck, Germany). Any ELISA titer ≥ 2 RU/ml was considered positive and the patients were further stratified on an "intermediate" titer (2-20 RU/ml) and an "high" titer (> 20 RU/ml) subgroup.

All biopsies were examined under immunofluorescence, light and electron microscopy by an experienced pathologist who, in addition to the evaluation of the glomerular pattern of injury, also assessed whether these biopsies showing a MN had features suggestive of secondary MN: immunofluorescence with full-house staining, vascular or tubular basement membrane staining, mesangial staining; light microscopy with mesangial and/or endocapillary hypercellularity; electron microscopy with subendothelial or mesangial deposits, vascular or tubular basement membrane deposits, tubuloreticular inclusions.

Results

Study population and anti-PLA2R serology characteristics

A total of 203 consecutive patients diagnosed with NS were included in the study and screened for the presence of anti-PLA2R antibodies. The study population had a mean age of 53 ± 13 years (38% of patients were over 60 years-old), with 61% of patients being males. Mean serum creatinine and median 24-h proteinuria were 1.84 ± 1.63 mg/dl and 6.8 g/24h (IQR:4.8-10.6), respectively. Thirty-seven patients (18.2%) had a positive work-up for secondary causes of nephrotic syndrome: 17 patients with viral infections (HBV/HCV), 6 patients with solid malignancies, 6 patients with hematologic malignancies, 10 patients with autoimmune disorders and 1 patient with a possible medication-associated NS (bisphosphonate). Of these, 3 patients had multiple concomitant causes of secondary NS (1 patient with HCV and sarcoidosis, 1 patient with HBV and acute myeloid leukemia, 1 patient with chronic myeloid leukemia and uterine cervix neoplasm).

When analyzing the distribution of anti-PLA2R serology we identified 67 patients with a "high" titer of anti-PLA2R antibodies (> 20 RU/ml) while 47 patients had an "intermediate" titer (2-20 RU/ml) (Figure 1). Patients with "intermediate" and "high" anti-PLA2R antibody titers had a better renal function at NS diagnosis compared to patients with negative serology (Table 1).

Table 1. Baseline characteristics of patients with nephrotic syndrome according to anti-PLA2R antibody titer.

Variable	PLA2R ab titer < 2 RU/ml	PLA2R ab titer 2-20 RU/ml	PLA2R ab titer > 20 RU/ml	P value
Number	89	47	67	
Age (years)	54 ± 13	52 ± 15	52 ± 13	0.58
Age ≥60 years (n, %)	37 (41%)	18 (38%)	24 (35%)	0.76
Gender (n, % males)	55 (61%)	26 (55%)	44 (65%)	0.53
Diabetes (n, % of pts)	24 (26.9%)	4 (8.5%)	5 (7.4%)	0.001
Secondary causes of NS (n,% of pts)	16 (18%)	10 (21.3%)	11 (16.4%)	0.99
Serum creatinine (mg/dl)	2.25 ± 2.15	1.56 ± 1.03	1.5 ± 0.89	0.03
eGFR (ml/min/1.73m^2)	50 ± 31	61 ± 31	61 ± 28	0.02
eGFR ≤ 60 ml/min (n, %)	58 (65.1%)	24 (51%)	29 (43.2%)	
Serum Albumin (g/dl)	2.7 ± 0.8	2.7 ± 0.5	2.7 ± 0.5	0.84
Total proteins (g/dl)	5.98 ± 1	5.48 ± 0.9	5.45 ± 0.9	0.002
24-h proteinuria (g/day)	7.4 (IQR:4.8-11.5)	6.2 (IQR:4.7-9)	7 (IQR:4.9-10.6)	0.36
Hematuria (cells/mmc)	21 (IQR:12-38)	32 (IQR:10-38)	27 (IQR: 10-56)	0.31

***Abbreviations**: ab, antibody; n, number; PLA2R, phospholipase A2 receptor; NS, nephrotic syndrome, eGFR, estimated glomerular filtration rate; IQR, interquartile range*.

On kidney biopsy examination, 95 patients had a diagnosis of MN, while 108 patients had other patterns of glomerular injury. In the subgroup of patients with "high" anti-PLA2R antibody titer, MN was diagnosed in 91% of cases (61 of 67 patients) with 6 patients showing other types of glomerular patterns of injury (2 patients with focal and segmental glomerulosclerosis, 1 with minimal-change disease, 1 with cryoglobulinemic glomerulonephritis, 1 with diabetic nephropathy and 1 with postinfectious glomerulonephritis). In the "intermediate" titer group, the histological diagnosis was more heterogenous, only 36% of patients showing a pattern of MN. Of patients with a histological diagnosis of MN, three had histological features of secondary MN (including one patient with a HBV infection) and all were negative for anti-PLA2R antibodies.

When analyzing the group of patients with MN, we identified 64.2% of them with "high" titer (n=61), 17.8% with "intermediate" titer (n=17) and 17.8% with negative serology (n=17, of whom 3 patients were positive for anti-THSD7A antibody)(Figure1).

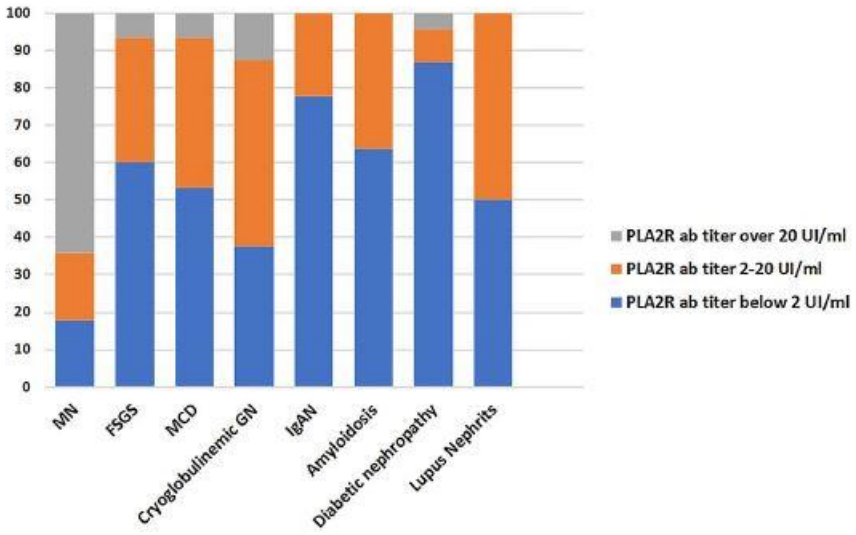

Figure 1.Distribution of anti-PLA2R antibody titre according to histological type.

Among those with MN, 19 patients (20%) had evidence of a possible secondary causes, of whom 9 patients had a "high" anti-PLA2R antibody titer (range: 86-818 RU/ml) and 3 patients had "intermediate" titers (range: 3-10 RU/ml).

Performance characteristics of anti-PLA2R antibodies to discriminate MN among different histological patterns of nephrotic syndrome

We then analyzed the performance characteristics of anti-PLA2R antibodies to discriminate MN among different histological pattern of NS at two different thresholds for defining a positive titer (2 RU/ml and 20 RU/ml). In the entire cohort, the area under the curve (AUC) was 0.83 (95% confidence interval [95%CI], 0.89-0.89; $p<0.001$) (Figure 2).

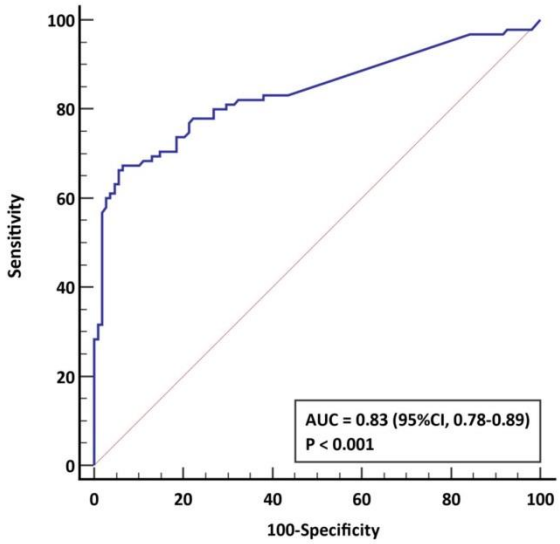

Figure 2. Diagnostic performance of anti-PLA2R antibody for pMN diagnosis in the entire cohort.

With a cut-off of 20 RU/ml, the anti-PLA2R antibodies had a 63% sensitivity (95%CI, 53-73%) and 94% specificity (95%CI, 88-97%) to discriminate MN from other causes of NS. Additionally, the PPV and NPV were 91% (95%CI, 82-95%) and 75% (95%CI, 69-79%).

When analyzing the posttest effect, we identified a large increase in the probability of MN if anti-PLA2R antibody titer was >20 RU/ml with a LR+ of 11.56 (95%CI, 5.2-25.2), but with a slight-moderate LR- of 0.38 (95%CI, 0.29-0.5). The inability to exclude MN if a negative test is due to the high number of patients with MN with a negative serology (n=34).

The overall accuracy of the test was 80.3% (95%CI, 74-85%) and the diagnostic odds ratio (DOR) was 30.42. Lowering the cut-off at 14 RU/ml, would slightly increase the sensitivity to 68% at the cost of an inferior specificity (88%). In this case, an additional 14 patients would have a positive serology, of whom only 4 showing histological features of MN.

We then stratified the patients according to age (≥60 years-old vs. < 60 years-old), kidney function (≤60 ml/min vs. > 60 ml/min) and work-up for secondary causes of NS (negative vs. positive). We identified that in younger patients, in those with preserved kidney function or with negative work-up for secondary causes the performance characteristics of anti-PLA2R were significantly improved. In this subgroup analysis, the

sensitivity of the test increased to 68-71% (compared to 64% in the entire cohort), the PPV increased to 93-95% and the LR+ was between 12.23 and 15.4, confirming that a positive test highly predicts a MN. Accordingly, the DOR was 39.41 in patients younger than 60 years, 40.15 in those with preserved renal function and 46.66 in those with negative work-up for secondary causes.

After multivariate adjustment, anti-PLA2R titer >20 RU/ml was the strongest predictor of MN (HR, 49.8; 95%CI, 17.02-144.4; p<0.001), while those with eGFR ≤ 60ml/min were 57% less likely to be diagnosed with MN compared to those with preserved kidney function(Table 2).

Table 2. Binary logistic regression analysis regarding clinical predictors of the diagnosis of primary membranous nephropathy in patients with nephrotic syndrome

Variable	**Univariate analysis**		**Multivariate analysis***	
	Odds ratio (95% CI)	**p-value**	**Odds Ratio (95% CI)**	**p-value**
Age (≥60 vs. <60 years-old)	1.28 (0.73-2.26)	0.38	2.4 (1.04-5.58)	0.04
Secondary cause of nephrotic syndrome (yes vs. no)	1.25 (0.61-2.55)	0.54	1.02 (0.38-2.7)	0.96
Diabetes mellitus (yes vs. no)	0.3 (0.13-0.71)	0.006	0.59 (0.19-1.81)	0.36
eGFR (≤60 ml/min vs. > 60 ml/min)	0.44 (0.25-0.78)	0.005	0.43 (0.19-0.97)	0.044
Anti-PLA2R antibody titer (vs. <2 RU/ml)	-	-	-	-
• **2-20 RU/ml**	2.4 (1.08-5.31)	0.03	2.17 (0.92-5.11)	0.07
• **>20 RU/ml**	43.05 (15.98-116)	<0.001	49.8 (17.02-144.4)	<0.001

Abbreviations: *NS, nephrotic syndrome; eGFR, estimated glomerular filtration rate; CI, confidence interval***.**

predictors of the histological diagnosis of MN.

Discussion

In this study, we confirmed that serum anti-PLA2R antibody screening in patients with NS is a useful method for the diagnosis of MN, especially in certain subgroup population.

In younger patients (less than 60 years-old), that have a preserved kidney function and a negative work-up for secondary causes a positive anti-PLA2R test highly predicts a diagnosis of primary MN.

The prevalence of anti-PLA2R antibodies in MN has been reported to vary between 50 and 88%, while a recent meta-analysis of 28 studies that explored the diagnostic test accuracy of anti-PLA2R antibodies showed a pooled sensitivity and specificity of 65% (95%CI, 63-67%) and 97% (95%CI, 97-98%), respectively (1,13).

In our cohort, at a cut-off of 20 RU/ml, the overall sensitivity and specificity of anti-PLA2R antibodies was 64% (95%CI, 53-73%) and 94% (95%CI, 88-97%), respectively. However, the comparison between different studies should be made with caution as different assays and cut-offs were used(9,18–22). For the ELISA method, the diagnostic test accuracy was evaluated for a cut-off of 2 RU/ml, 14 RU/ml and 20 RU/ml(13).

In our cohort, lowering the cut-off to 14 RU/ml or 2 RU/ml increased the sensitivity to 68% or 82% by trading for an inferior specificity of 88% or 66%, respectively.

In our cohort we identified 6 patients with an anti-PLA2R antibody titer higher than 20 RU/ml that had other patterns of glomerular injury (2 patients with focal and segmental glomerulosclerosis,1 with minimal-change disease, 1 with cryoglobulinemic glomerulonephritis,1 with diabetic nephropathy and 1 with postinfectious glomerulonephritis) that had anti-PLA2R antibodies with a titer ranging from 26 RU/ml to 190 RU/ml.

In the entire cohort, anti-PLA2R antibody screening had a diagnostic test accuracy of 80.3% (95%CI, 74-85%), a diagnostic odds ratio of 30.42 and LR+ of 11.56 (95%CI, 5.2-25.2), performance characteristics that are slightly inferior to those reported by Li *et al* in their recent meta-analysis (13).

This can be attributed to the high percentage of patients (35.8%) with negative serology and to the identification of glomerular pathologies other than MN with a high PLA2R titer. The relatively low sensitivity might bring limitations on the interpretation of test especially with a negative result.

Given these results we performed subgroup analysis stratifying patients by age, kidney function and the results of the initial work-up for secondary causes of NS. Accordingly, the sensitivity of the test increased to 68-71% (compared to 64% in the entire cohort), the PPV increased to 93-95%, the LR+ was between 12.23 and 15.4 and the DOR increased from 30.42 to 46.66, thus confirming that a positive test highly predicts a pMN in certain subpopulations.

Similarly, Bobart *et al* showed that in patients with anti-PLA2R antibodies, negative work-up for secondary causes and preserved kidney function (eGFR over 60 ml/min) a kidney biopsy did not provide additional diagnostic information as none of those

patients showed histological features suggestive of secondary forms or non-MN glomerular patterns of injury(14). Accordingly, this approach could be incorporated in a diagnostic algorithm but it need to be validated in independent cohorts.

Conclusions

In conclusion, we identified a prevalence of anti-PLA2R antibodies of 64% in patients with a histological diagnosis of MN. In younger patients that have a preserved renal function and a negative work-up for secondary causes of NS, a positive anti-PLA2R antibody test highly predicts an underlying primary MN. Thus, this is a useful biomarker that should be incorporated in the initial diagnostic work-up of patients with NS as in certain subgroups it may confidently allow to avoid the performance of a kidney biopsy.

In addition to their diagnostic role, sPLA2R-ab correlate strongly with disease activity and emerged as an important prognostic biomarker. A recent meta-analysis showed that patients with sPLA2R-ab had a poor clinical outcome with a lower clinical remission rate and a higher risk of renal failure (26).

Until the recently described antigens are fully validated and each distinct antigen-associated MN is clearly defined, the PLA2R-negative pMN should acknowledged as a distinct entity with a different clinical phenotype and outcome(26-29). Moreover, the most important predictors of clinical outcome in pMN remain uncertain.

Material and Methods Study 2

Study design and population

Accordingly, in our second study we sought to investigate the clinical outcome and to identify the independent predictors of clinical remission in a prospectively followed cohort of patients with pMN.

Primary MN was diagnosed by percutaneous kidney biopsy. All biopsies were examined under immunofluorescence, light and electron microscopy by an experienced pathologist. In addition to the characteristic histopathologic features of MN, the biopsies were also assessed for features suggestive of secondary MN. Additionally all patients underwent a systematic screening for secondary causes of pMN such as autoimmune disorders screening, hepatitis serology, age-appropriate malignancy screening, monoclonal gammopathy evaluation and medication history. Exclusion criteria were: age < 18 years

old, patients with prior immunosuppression (IS), patients with features suggestive of secondary MN, patients with a follow-up period of less than 24 months, patients with incomplete data or with missed visits, patients with a positive PLA2R serology but without a confirmatory kidney biopsy. Patients were treated in accordance with current KDIGO guidelines and the most recent randomized trials. The treatment was conducted at the discretion of the attending physician without any intervention.

Data collection

The follow-up visits were performed every three months in the first year and every 6 months thereafter. Baseline data was collected via electronic medical record review of patients at the time of NS diagnosis and included: age, gender, body mass index, history of smoking, presence of arterial hypertension, occurrence of thromboembolic events, serum creatinine, albumin and total proteins, lipid panel, serum fibrinogen, hemoglobin, serum IgG level, presence and titer of anti-PLA2R antibody, 24-h proteinuria and hematuria. Glomerular filtration rate (eGFR) was estimated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. MN stage was assessed by electron-microscopy according to Ehrenreich-Churg classification (26). Nephrotic syndrome (NS) was defined as 24-h proteinuria over 3.5 g/day in association with hypoalbuminemia.

Anti-PLA2R antibodies were determined by an ELISA assay (EUROIMMUN AG, Lübeck, Germany). A positive serology was defined as an ELISA titer ≥ 20 RU/ml. Patients were stratified by anti-PLA2R antibody status: PLA2R-negative pMN, PLA2R-positive pMN with a low titer (<200 RU/ml) or high titer (≥200 RU/ml).

Study endpoints

The primary outcomes evaluated during the follow-up period were the occurrence of immunological and clinical remission (either complete or partial remission). Complete remission (CR) was defined as proteinuria of no more than 0.3 g/day and a serum albumin of at least 3.5 g/dl. Partial remission (PR) was defined as a reduction in proteinuria of at least 50% from baseline to a level between 0.3 and 3.5 g/day. Immunological remission (IR) was defined as a decrease in anti-PLA2R antibody titer below 20 RU/ml.

Results

The study cohort had a mean age of 53 ± 12 years, 71% of patients were males and the mean eGFR was 62 ± 29 ml/min/1.73m2. The majority of patients had nephrotic syndrome (81.5%), the median level of 24-h proteinuria and the mean serum albumin being 8.7 g/d (IQR: 5.2-15.4) and 2.79 ± 0.65 g/dl, respectively. During the follow-up period, 20% of patients developed thromboembolic events.

In terms of PLA2R serology, 80% of patients had anti-PLA2R antibodies at diagnosis with a median level of 199 RU/ml (IQR: 100-320), 48% of these having an anti-PLA2R antibody level over 200 RU/ml.

Overall, the majority of patients received antiproteinuric treatment (97%) and an immunosuppressive regimen (92.3%). Most patients received cyclophosphamide-based regimens (47.7%), with 29.2% and 15.4% of the study cohort being treated with calcineurin inhibitors (CNI) or rituximab-based regimens, respectively, while 5 patients (7.7%) did not receive IS therapy.

Immunological remission was achieved in 63.5% of patients with a positive serology at a median time of 18 months (IQR:9.7-24). The renal response rate (complete and partial remission) was 73.8%, of which 32.2% of patients achieved a complete remission and 41.5% of patients achieved a partial remission at some point during the follow-up period. The median time to complete and partial remission was 18 months (IQR: 7.5-24) and 24 months (IQR: 12-24), respectively.

Subsequently, we analyzed the immunological and clinical response according to the baseline anti-PLA2R antibody status and the treatment regimen. In the study cohort, 13 patients had a PLA2R-negative pMN, while of those with PLA2R-associated pMN, 27 patients had a low anti-PLA2R antibody titer (<200 RU/ml) and 25 patients had a high anti-PLA2R antibody titer at baseline (≥200 RU/ml).

Patients with PLA2R-negative pMN had a better renal function (eGFR 79 ± 36 ml/min), higher serum albumin (3.1 ± 0.5 g/dl) and lower 24-h proteinuria (7.2 g/day, IQR: 3.4-11.1) compared to those with PLA2R-associated pMN (Table3). Additionally, the clinical outcome was better in patients with PLA2R-negative pMN compared to patients with PLA2R-positive pMN. These patients had a higher percentage of complete remissions (46.2%, compared to 33.3% in those with low anti-PLA2R antibody titer or 24% in those

with high anti-PLA2R antibody titer), a faster decline of 24-h proteinuria and lower time to complete remission (Table 3, Figure 3).

Table 3. Univariate analysis according to baseline anti-PLA2R antibody status.

Variable	Negative PLA2R	PLA2R ab <200 RU/ml	PLA2R ab >200 RU/ml	P value
Number of pts	13	27	25	
Age	54 ± 13	53 ± 13	51 ± 11	0.75
Gender (%M)	76.9%	63%	76%	0.58
BMI (kg/m^2)	31 ± 7.7	30.2 ± 4.8	30.6 ± 4.7	0.9
HTA (%)	100%	96.3%	92%	0.51
Smoking (%)	76.9%	55.6%	60%	0.42
Tromboembolic events (%)	7.7%	25.9%	20%	0.4
Serum creatinine (mg/dl)	1.15 ± 0.73	1.63 ± 0.94	1.33 ± 0.5	0.27
eGFR (ml/min)	79 ± 36	55 ± 27	61 ± 23	**0.05**
Serum Albumin (g/dl)	3.1 ± 0.5	2.8 ± 0.5	2.5 ± 0.6	**0.014**
Total Proteins (g/dl)	5.9 ± 0.9	5.6 ± 0.8	5 ± 0.7	**0.01**
Total cholesterol (mg/dl)	263 ± 104	304 ± 107	333 ± 115	0.18
Triglycerides (mg/dl)	187 ± 90	185 ± 101	223 ± 118	0.23
Fibrinogen (mg/dl)	597 ± 159	577 ± 155	642 ± 151	0.31
Hemoglobin (g/dl)	13.8 ± 1.8	13.8 ± 2.2	13.8 ± 1.6	0.96
24-h proteinuria (g/day)	7.2 (IQR: 3.4-11.1)	7.2 (IQR:4.2-14.4)	10.4 (IQR:7.1-17.4)	0.04
Hematuria (cells/mmc)	12 (IQR:5-26)	17 (IQR:9-40)	25 (IQR:15-38)	0.11
Serum IgG level (mg/dl)	474 (IQR:380-928)	470 (IQR:340-635)	470 (IQR:284-645)	0.42
Median PLAR2R ab titer (RU/ml)	0	100 (IQR:80-150)	320 (IQR: 320-467)	**<0.001**
MN stage (% of patients)				
• **I**	45.4%	8.3%	10.5%	**0.008**
• **II**	0%	33.3%	36.8%	
• **III**	27.3%	50%	52.6%	
• **IV**	27.3%	8.3%	0%	
Clinical response (% of patients)				
• **No response**	7.7%	25.9%	36%	0.4
• **Partial remission**	46.2%	40.7%	40%	
• **Complete remission**	46.2%	33.3%	24%	
Time to partial remission (mo)	18 (IQR:10.5-24)	12 (IQR:12-24)	18.5 (IQR:8.25-24)	0.95
Time to complete remission (mo)	6 (IQR:6-12.7)	18 (IQR:12-23.5)	23 (IQR:15.7-24)	**0.048**
Immunological remission (% of patients)	-	70.4%	56%	**<0.001**
Time to immunological remission (mo)	-	12 (IQR:9-18)	15 (IQR:8.25-18)	0.99
Antiproteinuric therapy (% of pts.)	92.3%	100%	96%	0.39
Immunosuppressive therapy (% of pts.)				
No IS	15.4%	7.4%	4%	0.56
Cyclophosphamide-based regimens	30.8%	51.9%%	52%	
CNI-based regimens	46.2%	22.2%	28%	
Rituximab-based regimes	7.7%	18.5%	16%	

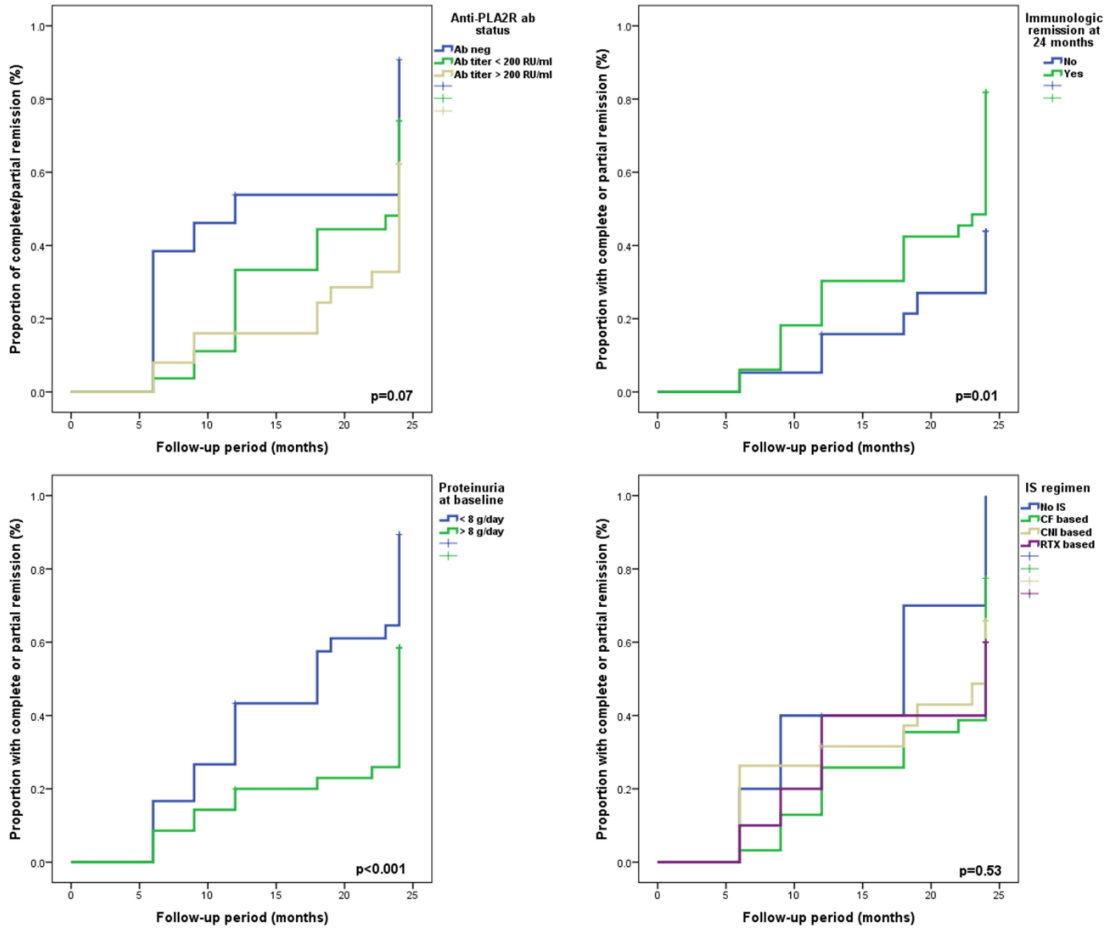

Figure 3. Cumulative proportion of patients with complete or partial remission according to PLA2R status, immunological remission at 24 months, baseline proteinuria and IS regimen.

In multivariate Cox regression analysis, patients with PLA2R-negative pMN had a 3.1-fold and a 2.87-fold higher chance for achieving a complete or partial remission compared to patients with high anti-PLA2R antibody titer or to all PLA2R-positive patients, respectively.

In terms of anti-PLA2R titer, patients with a low titer had a tendency for a milder clinical picture of pMN at diagnosis and for a better prognosis (higher percentage of immunological or clinical remissions) compared to those with a high titer (Table 3, Figure 3). However, after multivariate adjustment, we did not identify the anti-PLA2R antibody titer as an independent predictor of clinical remission.

We then analyzed the remission rates according to treatment regimen. In terms of baseline characteristics, patients treated with cyclophosphamide-based regimens had a

worse renal function (serum creatinine, 1.76 ± 0.91 mg/dl), higher 24-h proteinuria (median 11.4 g/day, IQR: 7-17.4) and lower serum IgG level (median 470 mg/dl, IQR: 327-618) compared to patients treated with other IS regimens or without IS, consistent with a more aggressive disease.

Regarding the immunological or clinical remission, we did not identify significant differences between different therapeutic interventions. However, in the treatment-naïve group (n=5), 66.7% of patients achieved a spontaneous immunological remission despite that the median level of anti-PLA2R antibody titer was similar to patients treated with different IS regimens. Additionally, despite having a similar immunological activity, these patients had a milder clinical phenotype with a lower 24-h proteinuria compared to patients treated with different IS regimens and a clinical response rate of 100% (complete or partial remission). By comparison, patients treated with rituximab-based regimens had the lowest clinical response rate and the highest level of proteinuria at the last follow-up visit .

In univariate and multivariate Cox regression analysis, the most important predictors of clinical remission were baseline proteinuria, the achievement of immunological remission at 24 months and the baseline negative serology (Table 4).

Patients with a baseline 24-h proteinuria of less than 8 g/day, with an immunological remission at 24 months or with a PLA2R-negative pMN had a 2.4-fold, 2.2-fold and a 2.87-fold, respectively, higher chance of achieving a clinical response (either complete or partial remission)(Figure 3). Renal function at diagnosis, type of therapeutic intervention or anti-PLA2R antibody titer did not predict the occurrence of clinical remission (Table 4).

Table 4. Univariate and multivariate Cox proportional hazard regression analysis regarding predictor of treatment response (partial or complete remission)

Variable	Univariate analysis		Multivariate analysis (model A)		Multivariate analysis (model B)	
	Hazard ratio (95% CI)	p-value	Hazard Ratio (95% CI)	p-value	Hazard Ratio (95% CI)	p-value
Serum Creatinine (for 1 mg/dl)	1.16 (0.79-1.7)	0.44	1.21 (0.76-1.93)	0.41	1.22 (0.76-1.95)	0.4
Serum Albumin (for 1 g/dl)	1.35 (0.89-2.04)	0.15	0.86 (0.48-1.52)	0.6	0.87 (0.5-1.52)	0.64
24-h Proteinuria (<8 g/d vs. ≥8 g/d)	2.31 (1.28-4.17)	0.005	2.38 (1.18-4.76)	0.01	2.4 (1.19-4.8)	0.01
Type of IS (vs. no IS)	-	-	-	-	-	-
• **CF-based regimens**	0.54 (0.18-1.57)	0.26	0.89 (0.29-2.73)	0.83	0.86 (0.28-2.62)	0.79
• **CNI-based regimens**	0.53 (0.17-1.65)	0.27	0.89 (0.26-2.98)	0.85	0.88(0.26-2.93)	0.83
• **RTX-based regimens**	0.44 (0.12-1.58)	0.21	0.64 (0.17-2.43)	0.51	0.64(0.17-2.43)	0.51
Immunological remission at 24 mo	1.46 (0.81-2.65)	0.2	2.25 (1.04-4.85)	0.04	2.26(1.05-4.87)	0.03
sPLA2R ab titer (vs. > 200 RU/ml)	-	-	-	-	-	-
• **<200 RU/ml**	1.37 (0.7-2.67)	0.35	1.13 (0.55-2.33)	0.73	-	-
• **Negative serology**	2.15 (0.98-4.69)	0.055	3.11 (1.13-8.53)	0.02	-	-
Anti-PLA2R ab (neg vs. pos)	1.18(0.92-3.58)	0.08	-	-	2.87(1.17-7.02)	0.02

Discussions

In this study, we have shown that patients with PLA2R-negative pMN had a better prognosis with an approximately 3-fold higher chance of achieving a clinical remission (either partial or complete remission) and a faster decline of proteinuria compared to patients with PLA2R-associated pMN. Additionally, baseline proteinuria seems to be a more important predictor of clinical outcome than sPLA2R-ab titer.

The threshold for sPLA2R-ab titer to define a high risk of progression is debatable and arbitrarily set at 150-200 RU/ml(27-31). In our cohort, patients with a low titer of sPLA2R-ab (<200 RU/ml) had only a tendency for a higher remission rate and a faster decline of proteinuria.

However, after multivariate adjustment, sPLA2R-ab titer was not identified as an independent predictor of clinical remission. On the other hand, patients with a negative serology had a 3-fold higher chance for a achieving a clinical remission (HR, 3.11; 95%CI, 1.13-8.53).

These findings suggest that PLA2R-negative pMN, possibly driven by one of the more recently described autoantigens, might represents a distinct entity.

In our study, antibody status at the end of follow-up, but not antibody titer at baseline, was associated with clinical remission (HR, 2.24 for immunological remission at 24 months; 95%CI, 1.04-4.85).

Similar findings were seen in the study of Bech et al in which 58% of patients that achieved an immunological remission at the end of therapy were in persistent clinical remission at 5 years, compared to none of the patients that remained immunologically active.

Conclusions

In conclusion, we identified a different clinical phenotype between PLA2R-positive and PLA2R-negative pMN. In such cases, the pathogenesis of MN is supposedly driven by a different autoantigen such as the recently described ones.

Patients with a negative serology had a 3-fold higher chance for a achieving a clinical remission (HR, 3.11; 95%CI, 1.13-8.53).

Patients with a baseline 24-h proteinuria of less than 8 g/day, with an immunological remission at 24 months or with a PLA2R-negative pMN had a 2.4-fold, 2.2-fold and a 2.87-fold, respectively, higher chance of achieving a clinical response (either complete or partial remission)

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1. **Roxana Jurubiță ,** Bogdan Obrișcă , Bogdan Sorohan , Camelia Achim , Georgia Elena Micu , Gabriel Mircescu , Gener Ismail. **Clinical Phenotypes and Predictors of Remission in Primary Membranous Nephropathy**. J Clin Med 2021 Jun 15;10(12):2624.doi: 10.3390/jcm10122624.

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1. **Roxana Jurubiță**, Bogdan Obrisca, Andreea Andronesi, Vlad Berbecar, Tudor Pașcanu, Gener Ismail. **Anti-phopholipase A2 Receptor Antibodies correlation with clinical and histopathological parameters reflecting disease activity in idiopathic membranous nephropathy.** Nephrology Dialysis Transplantation, Volume 31, Issue suppl_1, 1 May 2016, Pages i391–i392

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