The expression rate and prognostic value of phospholipase A2 receptor (PLA2R) in patients with idiopathic membranous glomerulopathy

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Abstract

Background and Objective: There are several studies around the world on the prevalence of phospholipase A2 receptor (PLA2R) autoantibodies (Anti-PLA2R) in patients with idiopathic membranous glomerulopathy indicating a wide heterogeneity in expression rate as well as its association with disease outcome. We aimed to determine the incidence of Anti-PLA2R by immunohistochemistry in biopsy of patients with membranous glomerulopathy and its relationship with clinical and pathological features and prognosis of patients.

Methods: This cross-sectional study was performed on 198 patients over 18 years of age with membranous glomerulopathy who underwent kidney biopsy from 2009 to 2019. Baseline information as well as data on disease outcome was extracted from patients' recorded files. Anti-PLA2R was tracked by immunohistochemistry staining in biopsied specimens.

Results: PLA2R was expressed in 88.9%. No association was revealed between PLA2R positivity and baseline parameters including gender, age, secondary etiology, positivity of serologic markers, disease stage, the type of glomerulosclerosis, concurrent tubulointerstitial nephritis (TIN) or acute tubular necrosis (ATN), intensity of pathological staining, and the mean of serum biomarkers related to kidney functional state. There was also found no association of PLA2R expression with disease outcome including death, disease recurrence, requiring hemodialysis and recovery rate.

Conclusion: Among our selected Iranian patients with membranous glomerulopathy, PLA2R expression is predictable in 88.9% of patients, but it seems that this marker has no prognostic role in these patients in our society.

INTRODUCTION

Membranous glomerulopathy as a specific autoimmune disease of the renal system is considered as one of the most important causes of nephrotic syndrome, especially in adults and Asian countries (1). The prevalence of disease has been increasing in the last ten years (2). About 80% of cases of membranous glomerulopathy are known as idiopathic nephropathy, while in the rest, the disease follows secondary causes such as infections, autoimmune diseases, cancers, or due to exposure to toxic drugs (3). The clinical course of the disease was different; one-third develop persistent proteinuria, with the majority developing end-stage renal disease (ESRD) within 5 to 15 years of onset, and one-third recovering spontaneously. Proteinuria is known as the hallmark of this disease. Although the evaluation of proteinuria severity is very valuable as a diagnostic method and determination of treatment regimen (4,5), this evaluation indirectly determines the severity of involvement in the disease as well as the autoimmune activity of the disease (6).

In 2009, for the first time, phospholipase A2 receptor type M or PLA2R was introduced as a major and target antigen in podocytes for membranous glomerulopathy. Patients with membranous glomerulopathy, particularly in the idiopathic type, experience an increase in the level of anti-PLA2R antibody in their serum, which was detectable in about 70% of patients (7). In other cases of membranous glomerulopathy, the expression of other types of specific antigens such as thrombospondin type A has been identified and detected (8). Also, reports of increased expression of thrombospondin type A antigen or antibody against it in about 10.5 to 16% of patients suffering membranous glomerulopathy with negative PLA2R (9,10).

In 2009, Beck et al demonstrated that the PLA2R plays a major antigen in patients with idiopathic membranous glomerulopathy. The Anti-PLA2R marker is known as a highly specific antibody for idiopathic membranous glomerulopathy because in patients with secondary membrane glomerulopathy, nephrotic syndrome has been sought for other causes and negative controls (1). This autoantibody is deposited in patients with idiopathic membranous glomerulopathy in the

subepithelial immune complex (12).

There are several studies around the world on the prevalence of PLA2R autoantibodies in patients with idiopathic membranous glomerulopathy. The methods for detecting the prevalence of this autoantibody include Western blot protein PLA2R, immunofluorescence assays (IFA), enzyme-linked immunosorbent assays (ELISA), immunohistochemical (IHC) staining and immunofluorescence on kidney biopsies (13).

Newer biomarkers are needed to reflect better disease autoimmune activity and determine the severity of disease involvement. Evidence suggests significant differences in the incidence of these antibodies in different communities and races, and therefore the prognostic and diagnostic value of these antibodies in diagnosing the severity of the disease in different communities has been very different. The aim of this study was to determine the incidence of Anti-PLA2R by immunohistochemistry in biopsy of patients over 18 years of age with membranous glomerulopathy and its relationship with clinical and pathological features and prognosis.

MATERIALS AND METHODS

This cross-sectional study was performed on patients over 18 years of age with membranous glomerulopathy who underwent kidney biopsy from 2009 to 2019 in Hasheminejad Hospital. In this study, patients with immunodeficiency state were excluded from the study.

First, by reviewing the patient file, information including demographic characteristics, primary or secondary disease course, severity of proteinuria, serum creatinine level, serum total protein concentration, the value of glomerular filtration rate (GFR), serum levels of complement and serological tests such as: (antineutrophil cytoplasmic antibodies (ANCA), VIRAL markers, elevated anti-double-stranded (ds) DNA antibody (DsDNA), antinuclear antibody (ANA)) along with outcome-related information including ten-year recurrence of the disease (proteinuria of more than 3.5 g in 24 hours) were extracted and entered in the prepared checklist. Then the patients' pathology reports were reviewed and histological findings were entered into a checklist. Paraffin blocks in the pathology department were isolated and after preparation of tissue incisions; immunohistochemical staining was performed on samples. The procedure was that all samples were stained with Anti-PLA2R rabbit monoclonal [EPR20483] (ab211573) at 1/2000 dilution. According to preparation protocol we used human renal tissue from a patient with renal clear cell carcinoma as positive control and human pancreatic tissue for negative control. The staining of PLA2R marker was considered positive in glomerular basement membrane region and other staining patterns were considered to be negative.

For statistical analysis, results were presented as mean ± standard deviation (SD) for quantitative variables and were summarized by frequency (percentage) for categorical variables. Continuous variables were compared using t test or Mann-Whitney test whenever the data did not appear to have normal distribution or when the assumption of equal variances was violated across the study groups. The categorical variables were compared using the Chi-Square test or Fisher's exact test if required. P values of ≤ 0.05 were considered statistically significant. For the statistical analysis, the statistical software SPSS version 23.0 for windows (IBM, Armonk, New York) was used.

RESULTS

At the beginning of the study, 200 samples were studied, of which 2 samples were not stained due to lack of tissue and therefore, finally, 198 patients were included in our study. Baseline characteristics are summarized in Table 1. The mean age of patients was 42.93±14.35 years and 54.5% were male. The frequency of secondary etiologies of disease was 16.7%. Serological markers were also positive in 98.0%. In terms of disease stage, stage I was reported in 22.2%, stage II in 74.7% and stage III in 3.0 %. Simultaneous TIN or ATN was identified in 19.7%. The pattern of kidney involvement in all patients was in the form of a glomerular basement membrane. In terms of staining intensity of immunofluorescence, grade I of staining was reported in 12.1%, grade II in 15.2%, grade III in 50.5%, and grade IV in 22.2%. Secondary histological changes were also reported in 12.1%.

Table 1: Baseline characteristics of study population

Male gender, %	108 (54.5)
Mean age, year	42.93±14.35
Secondary etiology, %	33 (16.7)
Mean urinary protein level	11.51±9.01
Mean serum creatinine level, mg/dl	1.40±1.01
Mean GFR level, ml/min	71.87±25.57
Mean serum total protein, mg/dl	4.94±1.13

Positive serum markers (ANA), %	194 (98.0)
Disease stage, %	
I	44 (22.2)
II	148 (74.7)
III	6 (3.0)
Mean percentage of IFTA, %	10.13±6.13
Sclerosing glomerulus, %	
Global	105 (53.0)
Segmental	93 (47.0)
Simultaneous ATN/TIN, %	39 (19.7)
Pattern of glomerular basement membrane involvement	198 (100)
Intensity of staining, %	
I	24 (12.1)
II	30 (15.2)
III	100 (50.0)
IV	44 (22.2)
Changes in secondary histology, %	24 (12.1)

During the follow-up period, complete recovery was found to be 50.5% considering cutoff value of 300 for proteinuria. Also, partially improvement was reported in 27.3% of these patients. The cases of non-response to treatment and recurrence of the disease was reported in 21.7%. The incidence of ten-year recurrence was equal to 2.5%. Needing hemodialysis was reported in 4.0% and death occurred in 4.0%.

In total, PLA2R was expressed in 176 cases (88.9%). In terms of PLA2R score, score 0 (negative) was detected in 11.1% figure 1, score 1+ (mild) in 15.7% figure 2, score 2+ (moderate) in 22.2% figure 3 and score 3+ (severe) in 51.0% figure 4. As indicated in Tables 2 and 3, no association was revealed between PLA2R positivity and baseline parameters including gender, age, secondary etiology, positivity of serologic markers, disease stage, the type of glomerulosclerosis, concurrent TIN or ATN, intensity of pathological staining, and the mean of serum biomarkers related to kidney functional state. There was also found no association of PLA2R expression with disease outcome including death, disease recurrence, requiring hemodialysis and recovery rate Table 4.

Table 2: The percentage of PLA2R expression according to baseline parameters

Parameter	Expression rate	P value
Gender		0.173
Male	91.7%	
Female	85.6%	
Secondary etiology		0.840
Positive	89.1%	
Negative	87.9%	
Positive serological markers		0.475
Positive	88.7%	
Negative	100%	
Disease stage		0.184
I	81.8%	
II	90.5%	
III	100%	

Sclerosing glomerulus		0.097
Global	92.4%	
Segmental	84.9%	
Simultaneous ATN/TIN		0.802
Positive	90.0%	
Negative	88.6%	
Intensity of immunoflorence staining, %		0.603
I	95.8%	
II	83.3%	
III	89.9%	
IV	85.7%	
Changes in secondary histology		0.155
Positive	79.2%	
Negative	90.2%	

Table 3: The mean quantitative parameters based on PLA2R expression

Parameter	PLA2R expression (+)	PLA2R expression (-)	P value
Mean age, year	43.31±14.51	39.91±12.92	0.296
Mean urinary protein level	7.60±5.79	6.39±4.34	0.354
Mean serum creatinine, mg/dl	1.39±0.11	1.40±0.25	0.976
Mean GFR level, ml/min	71.87±25.49	71.85±26.79	0.997
Mean serum total protein, mg/dl	4.91±1.12	5.15±1.21	0.513
Mean percentage of IFTA, %	10.51±1.27	5.62±0.62	0.199

Table 4: The percentage of PLA2R expression according to disease outcome

Parameter	Expression rate of PLA2R	P value
Complete recovery (cutoff: 300)		0.960
Positive	89.0%	
Negative	88.8%	
Partial recovery (cutoff: 300)		0.612
Positive	87.0%	
Negative	89.6%	
No response to treatment		0.670
Positive	90.7%	
Negative	88.4%	
10-year recurrence		0.197
Positive	100%	
Negative	88.6%	
Hemodialysis		0.307
Positive	100%	

Negative	88.4%	
Death		0.307
Positive	100%	
Negative	88.4%	

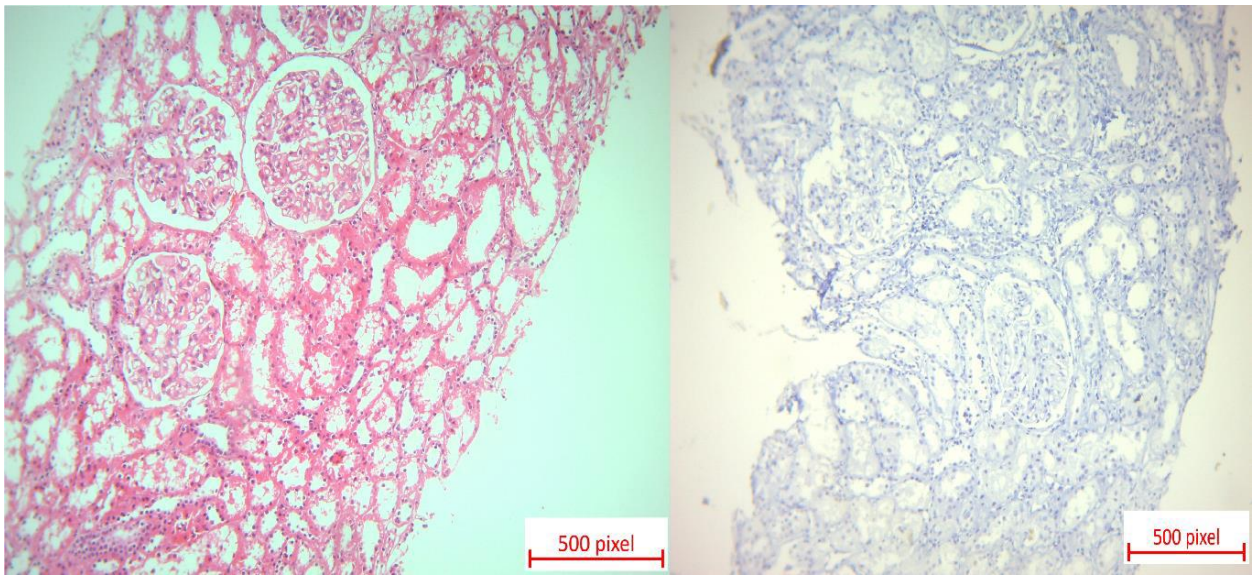

Figure 1. Slides of a renal biopsy specimen from a case with secondary MGN histology.
A: Hematoxylin and Eosin (H&E) staining. B:immunohistochemical staining for PLA2R score (0) IHC staining is negative in subepithelial region.

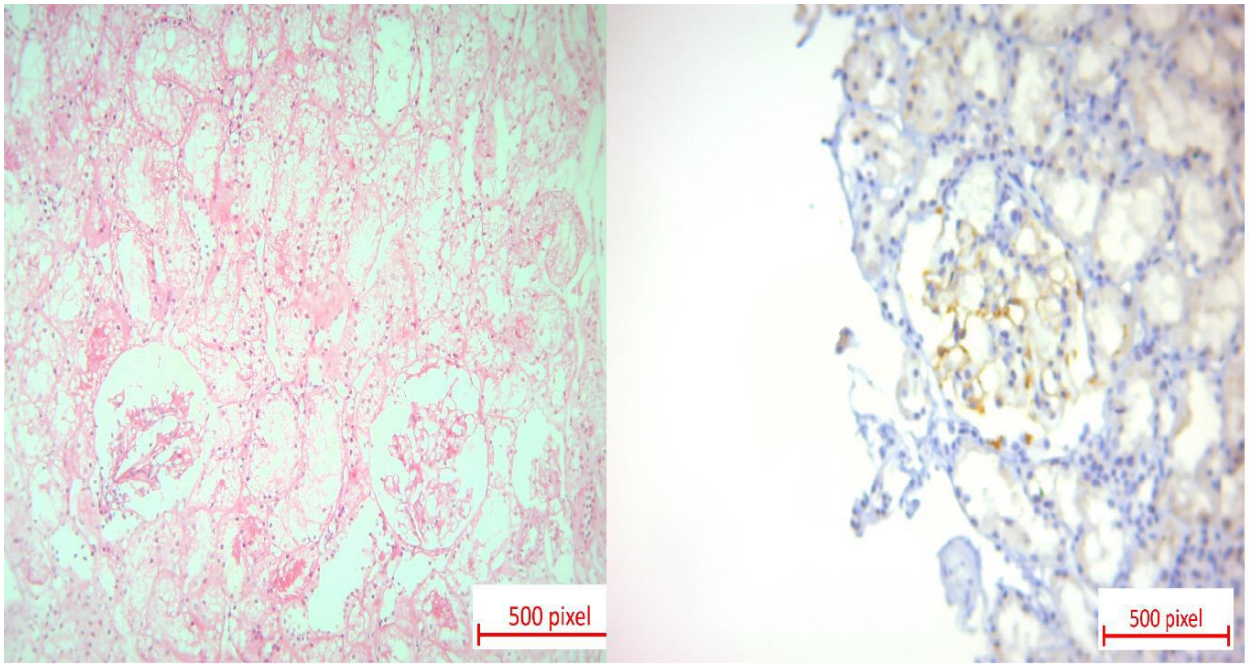

Figure2. Slides of a renal biopsy specimen from a case with MGN histology. A: Hematoxylin and Eosin (H&E) staining. B: immunohistochemical staining for PLA2R score (1+) , IHC staining shows faint subepithelial deposits.

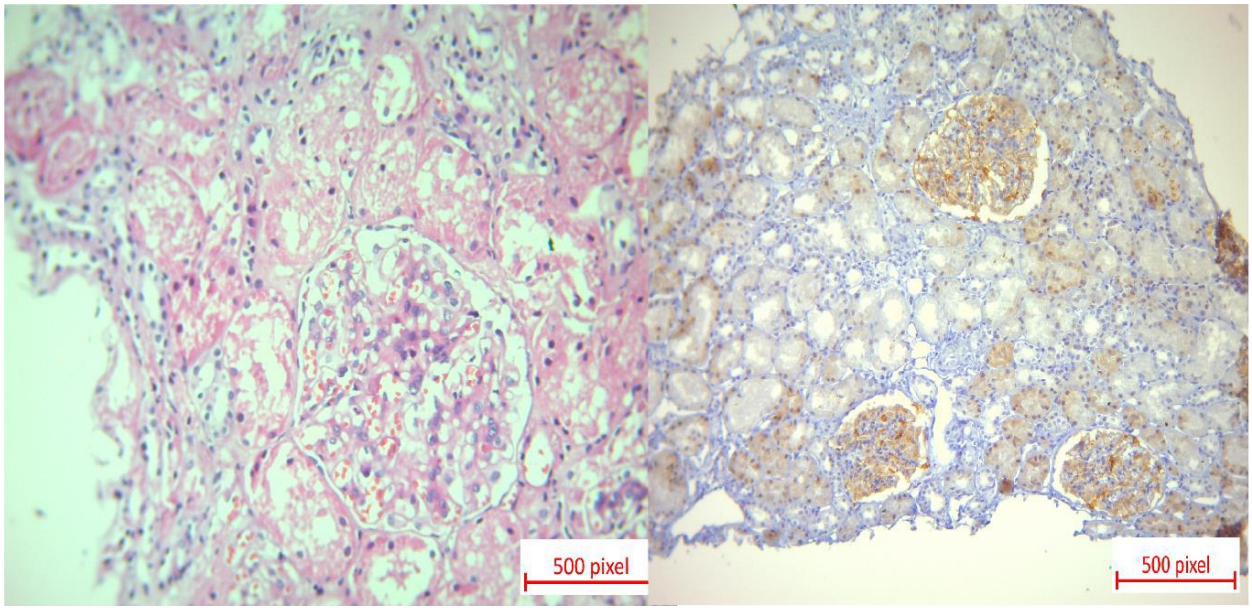

Figure 3. Slides of a renal biopsy specimen from a case with MGN histology. A: Hematoxylin and Eosin (H & E) staining. B: immunohistochemical staining for PLA2R score (2+), IHC staining shows moderate subepithelial deposits.

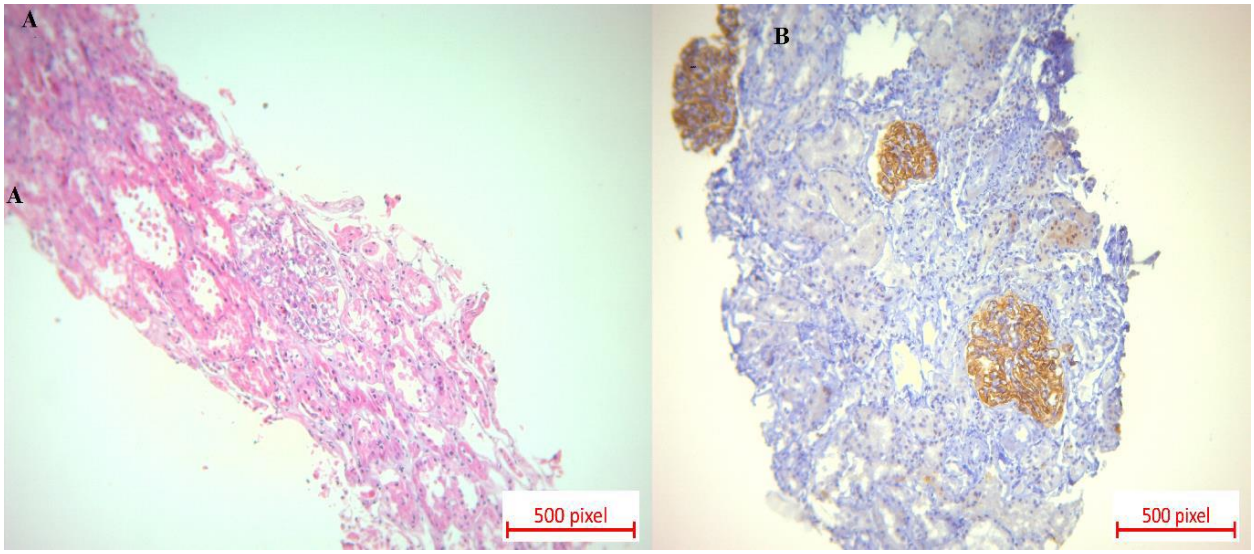

Figure 4. Slides of renal biopsy specimen from a case with MGN. A: Hematoxylin and Eosin (H&E) staining, B: immunohistochemical staining for PLA2R score (3+), IHC staining shows diffuse and strongly subepithelial deposits.

DISCUSSION

Evaluating clinical progress as well as predicting the long-term clinical consequences of patients with membrane glomerulopathy is critical due to the lack of significant improvement in these patients and plays an essential role in the treatment planning of these patients. In this regard, evaluation of renal function in terms of proteinuria severity or renal clearance status can be very helpful; however, these findings are not very specific in these patients. The study of genomic variants or more specific antigens to assess the prognosis of the disease has recently received special attention. An increase in the level of anti-PLA2R antibody in the serum of these patients has been shown. In some recent studies, the relationship between this marker and histopathological features of patients, clinical manifestations and therapeutic prognosis have been even shown in the long run. What we did in the present study was to determine the incidence of anti-PLA2R in adult patients with membranous glomerulopathy and its relationship with pathological and clinical characteristics and prognosis of patients in a selected population of Iranian patients.

What can be seen in the findings of the present study was, firstly, the lack of a significant relationship between the expression of this marker and the underlying characteristics of patients, including demographic characteristics as well as histopathological features of the disease. Additionally, considering the various aspects of prognosis including complete recovery, partial recovery, disease recurrence, the need for hemodialysis due to end-stage renal failure or mortality, there was found no a significant relationship between PLA2R expression with these events. This could indicate the fact that in different societies,

different and heterogeneous roles of PLA2R expression as a prognostic factor in patients with membranous glomerulopathy can be expected. In our chosen Iranian society, expression of PLA2R gene or increase in anti-PLA2R antibody titer in these patients can not have a prognostic role and therefore should still be sought in other histopathological or genomic parameters. Of course, it is necessary to state a few points that affect the accuracy and reliability of our results. First, the sample size of the study to express such a role and relationship could be small, and a wider community should be considered, taking into account racial and genetic variants. Moreover, considering the high stages of the disease in most patients gives more reliable results, however, a significant proportion of our patients had intermediate or lower stages of the disease and a small proportion of patients had a poor prognosis. In fact, complete or partial recovery was reported in a large proportion of our patients.

However, what shows the consistency of our study with previous studies was the high expression of PLA2R marker in Iranian patients with membranous glomerulopathy, which was also reported in other communities. In the study by Zhang et al, the PLA2R expression rate in these patients was 85.4%, which was very close to our rate. But in their study, 24-hour urinary protein and blood pressure were significantly higher in patients with PLA2R-related but low serum albumin levels were reported. But as in our study, there was no significant difference between the two groups with and without expression of this marker in terms of age, sex, serum creatinine level, eGFR, pathological stage of the disease, however, the rate of proteinuria improvement was lower in PLA2R-related patients than in the other group (14). In the study of Tian et al, the expression of PLA2R was 71.7% and unlike our study, the relative and complete recovery rate was lower in patients with PLA2R (15). In the study by Kumar et al., 60% of infected children had antibodies against PLA2R. Simultaneously with decreasing anti-PLA2R titer, the severity of proteinuria in patients also decreased (16). In the study of Akiyama et al., PLA2R antibody was detected in the serum of 53% of patients with idiopathic type and none of the secondary types (17), which indicate that the expression of this marker is mainly dependent on the pathological type of the disease. In another study in Iran by Ardalan et al, IFA anti-PLA2R antibody was found in 74% of idiopathic patients and none of the secondary types. In this study, no correlation was found between the amount of proteinuria and the titer of this antibody (18), which was completely consistent with our findings. Therefore, the prognostic role of PLA2R expression in patients with membrane glomerulopathy should be emphasized on the genetic and racial characteristics of patients.

CONCLUSION

As a final result, among our selected Iranian patients with membranous glomerulopathy, PLA2R expression is predictable in 88.9% of patients, but it seems that this marker has no prognostic role in these patients in our chosen society.

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