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Journal of Clinical Research and Reports

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Research Article

Which Immunosuppressive Therapy should be used in Primary Membranous Glomerulonephritis?

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Received date: October 19, 2024; **Accepted date:** December 21, 2024; **Published date:** December 27, 2024

Citation: Busragul Aksoy, Nedim Yilmaz Selcuk, İbrahim Guney, Siddika Fındık, (2024), Which Immunosuppressive Therapy should be used in Primary Membranous Glomerulonephritis?, *J Clinical Research and Reports*, 17(3); **DOI:10.31579/2690-1919/423**

Abstract

Background:

Different results have been reported on immunosuppressive treatments in patients with primary membranous nephropathy. In recent years, it has been determined that antiPLA2R antibodies can be used for the diagnosis of primary membranous glomerulonephritis. The aim of this study was to investigate the treatment responses of patients with primary membranous glomerulonephritis determined by the presence of PLA2-R antibody.

Patients and Methods:

Sixty patients (M:29, F:31) with membranous glomerulonephritis were retrospectively investigated. The presence of glomerular PLA2R antibodies were investigated by immunohistochemical method from the pathological specimen. Those patients were treated with immunosuppressants (methylprednisolon, cyclophosphamide, cyclosporine, azathioprine), and angiotensin-converting enzyme inhibitor (ACE-I) or angiotensin II receptor blocker (ARB). The treatments were carried out with different combinations.The success of the treatment was defined as complete (<0.3g/day), partial (<3.5g/day or at least 50% reduction) or unresponsive (insufficient reduction) according to the reduction in proteinuria. In the patients, progression risks (low, moderate, high) were divided according to proteinuria and creatinine clearance.The results of immunosuppressive treatments were statistically analyzed, and $p<0.05$ was accepted significant.

Results

Glomerular PLA2R antibodies were positive in 50 cases (87.7%), negative in 7 cases (12.2%). The mean duration of treatment of the patients was 23 months (6-58 months). With the treatment of PLA2R antibody-positive patients, 29% developed complete remission, 56% partial remission, and 15% did not respond. Complete remission was achieved only by steroid plus cyclophosphamide or cyclosporine combinations. The overall response (complete plus partial) rates were similar (respectively, 91% and 89%) in cyclophosphamide or cyclosporine treatment with steroids.

The reduction rates in proteinuria were 70.6% in patients using cyclophosphamide, steroid, and ARB, and 79.5% in patients using cyclosporine, steroid, and ARB; there was no difference between them (P=0.67). In patients with positive PLA2R antibodies, the risk of progression was low in 25%, moderate in 29%, and high in 46%. When the treatment responses were examined, a lower rate of complete remission and higher partial remission were found in the high-risk group than in the low-risk group. However, overall response rates were not different in risk groups.

Conclusion

In primary membranous glomerulonephritis, only cyclophosphamide or combinations of cyclosporine and steroids provided complete remission. The complete plus partial response rates were similar in all risk groups.

Keywords: primary membranous glomerulonephritis; immunosuppressive drugs; treatment of risk groups; complete remission

Introduction

The most common primary glomerulonephritis in adults has been reported to be membranous nephropathy (IMN) [1-3]. Hence, detailed studies are performed on the pathogenesis, follow-up, and treatment of IMN. In recent years, it has been accepted that autoantibodies developed against phospholipase A2 receptors in glomerular podocytes play a role in the pathogenesis of IMN. In a significant meta-analysis, detection of PLA2R antibodies in serum and glomeruli is reported that is a good diagnostic tool in differentiating idiopathic and non-idiopathic membranous GN [4].

The presence of PLA2R antibodies in biopsy samples for the diagnosis of IMN has been demonstrated to be more sensitive than the serological test [5,6]. Anti-PLA2R antibodies circulating in the serum are detected in most patients with renal deposits, and false-negative results are very rare [5].

A definitive effective treatment method for IMN has not been determined. Non-immunosuppressive therapy or immunosuppressive therapies (e.g., steroid, cyclosporine, tacrolimus, cyclophosphamide, rituximab) are commonly used in the treatment. The present study aimed retrospectively to investigate the effects of immunosuppressives and glucocorticoid treatments, which are frequently administered to treat patients with primary membranous glomerulonephritis by biopsy.

Patients and Methods

Sixty patients (M:29, F:31), diagnosed membranous glomerulonephritis by kidney biopsy were examined for the presence of glomerular phospholipase A2 receptor antibodies by immunohistochemical staining. Permission was obtained from the local ethic committee for the study. The examinations in the files of the patients were reviewed retrospectively.

One lysine slide section was taken from paraffin blocks of 60 patients for immunohistochemical staining. Sections were kept in an oven for one night and placed on the Ventana Benchmark XT instrument. ABCAM anti-phospholipase A2 antibody (ABCAM ab58375) was used as the primary antibody, diluted 1/100. It was a Rabbit polyclonal IgG antibody against phospholipase A2. An ultra-View Universal DAB detection kit (Ventana Medical Systems, Inc. Ventana) was used as a secondary antibody. This kit is an indirect biotin-free system to determine rabbit primary antibodies. The glasses coming out of the device were washed with detergent water, dried, rinsed with alcohol, placed in xylene, and covered with balsam. A single pathologist evaluated slides stained with Hematoxylin Eosin and anti-phospholipase-A2 for positivity and negativity with an Olympus BX51 model light microscope.

Immunosuppressive treatments applied to the patients were reviewed retrospectively. Therefore, nephrologists administering the treatment had no prior knowledge of the study.

Patients were divided into those with and without glomerular PLA2 antibodies. Also, they were divided into groups according to their progression risks. Patients with proteinuria of <4g/day and creatinine clearance within normal limits were considered to have a low progression risk, patients with proteinuria of 4-8g/day and creatinine clearance within normal or borderline limits as a moderate progression risk, and patients with proteinuria of >8g/day and/or reduced creatinine clearance as a high progression risk.

Complete remission was considered in patients whose proteinuria decreased to <0.3 g/day with treatment, partial remission in patients whose proteinuria decreased to <3.5g/day or at least 50%, and no response in patients who did not meet these criteria.

In statistical analysis, normal distribution was investigated by Skewness and Kurtosis or D' Agustino-Pearson tests. Values that did not show normal distribution were compared with the Mann-Whitney U test in independent groups and the Wilcoxon test in the dependent groups. Chi-square test or Chi-square test with Yate's correction for ≤5 patients or Fisher exact test for ≤3 patients were used for determining the differences between categorical data. Results were considered statistically significant, if the p-value was <0.05.

Results

The mean age of the patients was 50.2±16.9 years (18-80 years). At the time of admission, 45 (75%) of the patients had edema. PLA2R antibodies were determined to be positive (88%) in 50 patients. Tissue was insufficient for immunohistochemical evaluation in 3 patients for PLA2R antibody. PLA2R antibody was negative (12%) in 7 patients. 3 of the 60 patients had tissue insufficiency to detect the presence of PLA2R antibodies. In 2 of the 50 positive patients, information about the treatment could not be reached.

The patients were treated using immunosuppressive drugs, angiotensin-converting enzyme inhibitor (ACE-I) or angiotensin II receptor blocker (ARB). The mean treatment time was 23(6-58) months. As the immunosuppressive treatment, methylprednisolone was administered to 38 patients (63%) for 6-35 months, cyclosporine to 21 patients (35%) for 6-30 months, cyclophosphamide to 21 patients (35%) for 6-28 months, azathioprine to 7 (13%) patients for 6-31 months, and mycophenolate mofetil (MMF) to 1 patient (1.6%). In other words, patients mainly used steroids, cyclophosphamide or cyclosporine. The doses were methylprednisolone 1mg/kg/day per oral(po), cyclosporine 3-4mg/kg/day po (blood level: 100-200mg), cyclophosphamide 600mg/m2 body surface area (maximum 1g) intravenous (iv) infusion once a month. Treatment was continued for at least six months. Doses were reduced for treatments longer than six months. The cumulative dose of cyclophosphamide was <10g. To prevent urinary system adverse effects, 400mg of Uromitexan as IV infusion (at least in 15 minutes) was administered to the patients who had cyclophosphamide. If the adequate response was not achieved with cyclophosphamide, it was changed to cyclosporine. Azathioprine was similarly administered sequentially.

ACE-I or ARB was given to 41 patients (68%) at standard doses for 6-42 months. No severe adverse effects were determined to discontinue during drugs treatments. The important results of treatment in all patients (n=55) are summarized in Table 1. After treatment, urea, creatinine, and serum albumin values increased, eGFR and proteinuria decreased (14% and 89%, respectively).

Laboratory Parameters	Pre-treatment Mean ±SD	Median	Post-Treatment Mean ±SD	Median	P
Urea (mg/dL)	34.4±20	27	44±34	32	0.01
Creatinine (mg/dL)	0.89±0.54	0.75	1.26±1.32	0.9	0.003
eGFR(mL/min/1.73m2)	105.6±41.7	106	85±40.8	91	<0.001
Proteinuria (g/24hours)	8.5±5.9	7.3	3.4±5.1	0.8	<0.001
Serum Albumin(g/dL)	2.7±0.7	2.6	3.8±0.7	4	<0.001

Table 1: Effects of treatments on some important laboratory results in patients.

Proteinuria, GFR, and serum albumin levels were analyzed in used immunosuppressive combinations. The effects of the treatment modalities used in PLA2R positive patients(n=48) are summarized in Table 2.

	Proteinuria (g/24 h.)	GFR (mL/min.)	Albumin (g/dL)	Duration (months)
Cyclops. + Steroid +ARB(n=11)				
Pre-treatment	7.2±4.3	97±38.5	2.6±0.5	
Post-treatment	1.7±2.3	72.5±31	4.0±0.3	24±15
Cyclophosph +Steroid +ARB(n=9)				
Pre-treatment	8.2±3.6	95±25	2.3±0.6	
Post-treatment	2.6±3.1	88±26	3.4±0.7	19±7
Cyclophosph., Cyclosp., Steroid, ARB(n=7)				
Pre-treatment	10	104	2.2	
Post-treatment	2.1	72	4.1	27
ACEi or ARB(n=9)				
Pre-treatment	4.9±4.4	98.6±29.3	3.6	
Post-treatment	3.1±3.6	87.8±26	4.0	18.8±12.7
Azathioprine, Cyclophosphamide, cyclosporine, Steroid, ARB(n=2)				
Pre-treatment	9,1	76	1.9	
Post-treatment	1,3	62	3.8	47
Cyclophosphamide, Steroid(n=1)				
Pre-treatment	5.2	102	3.1	
Post-treatment	0.4	109	3.8	32
Steroid, ARB(n=3)				
Pre-treatment	10.7	115	2.8	
Post-treatment	0.28	100	4.4	31
Cyclophosphamide(n=1)				
Pre-treatment	16	81	1.6	
Post-treatment	7.8	85	3.6	35
Cyclosporine(n=1)				
Pre-treatment	1.1	129	4.1	
Post-treatment	0.8	107	3.9	30
Steroid, ARB/ACEi(n=4)				
Pre-treatment	7.2	104	3.1	
Post-treatment	0.8	88	4.4	24

Table 2: Treatment modalities and effects used in PLA2R positive patients (mean±SD or median)

Proteinuria decreased (t=-4.94, p<0.01), GFR did not change (P=0.46), serum albumin increased (t=4.5, p<0.002) in patients using cyclophosphamide, steroid and ARB combination (group A). Proteinuria decreased (t=-6.43, p<0.001), GFR decreased (t=-2.96, p<0.01), serum Albumin increased (t=5.45, p<0.0003) in patients using cyclosporine, steroid and ARB (group B). Proteinuria decreased (P=0.03), GFR decreased (P=0.04), serum albumin not increased (p=0.07) in patients who received cyclophosphamide, steroid, cyclosporine and ARB (group C).

The mean reduction in proteinuria were 70.6% (70.6±33.1) in group A, were 79.5% (79.5±16) in group B, were 78% (median: 78) in group C. The rate of decrease in group A was not different from group B (Z=0.42, P=0.67) and group C (Z=0.81, P=0.41). The decrease rate in group B was not different from group C (t=0.78, p=0.44).

Different combinations made between steroid plus azathioprine and cyclosporine or cyclophosphamide were evaluated as total (n=7). It was determined 91% (median=91) reduction in proteinuria. No difference was found between azathioprine users and group A (Z=0.57, P=0.56) or group B (Z=0.42, P=0.67) or group C (Z=0.63, P=0.52) or ACEi/ARB (Z=1.58, P=0.11).

The decrease rate was 46.8% (46.8±38.8) in those using only ACEi or ARB (n=9). The rates of reduction in proteinuria in ACEi/ARB users were lower than group C (t=2.54, p=0.02) and were not different from group B (t=1.39, p=0.18) and group A (Z=0.95, p=0.34).

Consequently, it was determined that different modifications made with immunosuppressants in treating primary membranous nephropathy with positive PLA2R antibodies reduced proteinuria at similar rates. Complete remission developed in 26.2±9.3 months in 14 (29%) of the PLA2R antibody-positive patients. Complete remission was 33.3% in cyclophosphamide, steroid, and ARB users, and 36.3% in cyclosporine, steroid, and ARB users; there was no difference between them (X^2=0.108, P=0.741).

Partial remission developed in 6 patients (55%) using cyclosporine, steroids, and ARBs, the reduction rate in proteinuria was 70%, and 5 (56%) patients using cyclophosphamide, steroid, and ARB developed partial remission and the reduction rate in proteinuria was 64%. The rates of reduction in proteinuria were not different (Z=0.18, P=0.85). When the overall response (complete plus partial remission) was examined together, it was 91% in those using cyclosporine, steroid, and ARB, and 89% in those using cyclophosphamide steroid and ARB. It was not different (p>0.05).

Patients treated with PLA2R antibody positive were divided into groups regarding progression risk. 12 (25%) were in the low-risk group, 14 patients (29%) were in the moderate-risk group, and 22 patients (46%) were in the high-risk group. That is, approximately half of the patients were in the high-risk group. The treatment responses of these patients were analyzed according to the risk of progression (Table 3).

Risk Groups	Complete Remission	Partial Remission	No Response
Low (n=12)	59%	25%	16%
Moderate (n=14)	29%	50%	21%

High (n=22)	14%	68%	18%

Table 3: Response to treatment of PLA2R-positive patients disaggregated by progression risk

Complete remission rates of the high-risk group were lower than the low-risk group (X^2=0.015; p<0.05) and similar to the moderate risk group (X^2=0.39; p>0.05). The low-risk and moderate-risk groups were determined to be similar (X^2=1.28; p>0.05).

Partial remission rates of the patients in the high-risk group were higher than those in the low-risk group (X^2=0.029, p<0.05). Partial remission rates of the patients in the moderate and high-risk groups were similar (X^2=1.19; p>0.05).

The overall response rates (84% and 82%) of the low and high-risk groups were similar (p>0.05). It was similar (p>0.05) in the moderate (79%) and high-risk groups (82%). As a result, complet remission rates were low in high-risk group than low-risk group. Complete remission rates were similar in moderate end low-risk groups. Partial remission rates were high in high-risk group than low-risk group. However, the overall response rates to treatment (79-84%) were not different in all risk groups. The rate of unresponsiveness to treatment was not different according to the risk of progression (16-21%).

Discussion

In the diagnosis of membranous glomerulonephritis, the presence of PLA2R antibodies in biopsy samples was more sensitive than the serological test [7]. In our study, PLA2R antibodies were determined as 87.7% in the biopsy samples. It was also reported as 76.6% in Spain [5]. IV cyclophosphamide and steroids in the treatment of IMN is found beneficial in achieving remission [8]. In other a study, the complete remission rate was 68.8% and 81.2% at the 15th month in patients used IV cyclophosphamide (500-750mg/m2) and oral prednisone [9]. In our study, monthly pulse cyclophosphamide plus oral metilprednisolon were given, and a high overall remission rate (79-84%) were observed. Some studies have suggested that cyclic cyclophosphamide and glucocorticoid (modified Ponticelli protocol) therapy are more beneficial than others [10-12]. In a meta-analysis including 21 clinical studies, calcineurin inhibitors were suggested as an alternative to cyclophosphamide in patients with IMN [13].

A particularly recommended treatment plan could not be presented in the meta-analyses on the subject. In 36 randomized controlled meta-analysis studies (n=2018) comparing immunosuppressive treatments in idiopathic membranous nephropathy, 11 types of treatments were examined. Among the immunosuppressives, only cyclophosphamide and chlorambucil were found to reduce the risk of mortality and end-stage renal disease but carry a significant risk of toxicity. It was reported that tacrolimus and cyclosporine increase the likelihood of remission in proteinuria, but their effects on kidney failure are unknown [14].

The effects of IMN treatments with cyclosporine (CSA), tacrolimus (TAC), or cyclophosphamide (CTX) combined with steroids for 48 weeks have been revealed to be no different. At the end of 48 weeks, response rates were 74% with CsA, 84% with TAC, and 82% with CTX [15]. In our study, the cumulative remission rates of cyclosporine or cyclophosphamide treatment with steroids for an average of 23 months were similar (91% and 89%). The KDIGO 2021 guideline for glomerulonephritis treatment has recommended planning the treatment according to the risk of progression for IMN [16].

In our study, after using glucocorticoids together with cyclophosphamide or cyclosporine for an average of 23 months, the complete remission rate was lower in high-risk patients than in moderate and low-risk patients.

Complete remission was high in the low-risk group. However, the overall response rates to treatment were not different in all risk groups. By the Guideline, cyclophosphamide and cyclosporine were similarly effective in moderate and high-risk groups. In two randomized controlled studies (GEMRITUX and MENTOR studies), Rituximab treatment has been suggested to be more effective than other immunosuppressives [17]. However, in another randomized controlled RI-CYCLO study, rituximab was compared with cyclic cyclophosphamide plus steroid. The cumulative incidence of complete and partial remissions at 24 months was similar (80% in both groups) [18]. In primary membranous nephropathy, complete remission was not achieved in most patients with Rituximab monotherapy. In conclusion, it was revealed that different combinations with immunosuppressants reduced proteinuria at similar rates. The cumulative remission rates of treatment with cyclosporine or cyclophosphamide combined with steroids were not different in low, moderate, and high-risk patients. These findings show that all patients with primary membranous nephropathy should be treated with immunosuppressants.

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DOI:10.31579/2690-1919/421