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Temporal Association Between PLA2R Antibodies and Clinical Outcomes in Primary Membranous Nephropathy

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Introduction: Autoantibodies to M-type phospholipase A2 receptor (aPLA2R) are seen in two-thirds of patients with primary membranous nephropathy (PMN) and are associated with disease activity. However, the precise temporal dynamics between the presence and amount of aPLA2R in circulation, as well as the clinical activity, are not known. We evaluated the temporal association between disease activity and serum aPLA2R during and after treatment in PMN.

Methods: The study included all patients with PMN and elevated aPLA2R who were started on immunosuppressive therapy for persistent nephrotic syndrome at a single center between December 2014 and December 2015. Serum samples were tested for aPLA2R at baseline and at monthly intervals for 6 months. Clinical details were collected monthly for 9 months. Serological remission was defined as negative aPLA2R in 2 consecutive samples. Clinical remission was defined by standard criteria.

Results: A total of 30 patients with PMN were studied. Of these, 28 (93%) had elevated levels at baseline, whereas 2 (7%) became positive after 1 month. The mean age was 33.2 $\pm$ 1 (range, 13–52) years. Median baseline aPLA2R titer was 163.41 (range, 70–291.01) RU/ml. A total of 24 patients (80%) achieved serological remission by 6 months. Among all the serological responders, 54% had achieved negative aPLA2R by the end of the first month. Clinical remission was observed in 20 patients (67%). Serological and clinical remission were noted at 2.7 $\pm$ 1.71 and 5.05 $\pm$ 2.64 months, respectively.

Discussion: In patients with aPLA2R-associated PMN, reduction in circulating aPLA2R precedes clinical remission. Persistence of aPLA2R at the end of therapy is associated with clinical resistance.

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Beck *et al*. reported autoantibodies to M-type phospholipase A2 receptor (aPLA2R) in 70% of patients with primary membranous nephropathy (PMN).[1] A report by Debiec and Ronco of serological positivity in more than 50% of PMN cases confirmed this finding.[2] Subsequent reports documented seropositivity in 67% to 82% of patients with PMN.[3,4] A minority were found to have no circulating antibody but showed glomerular staining, and vice versa.[5] The current understanding is that more than 80% of PMN is aPLA2R related.[3]

Hoxha *et al*.[6] carried out a prospective observational study in 133 subjects with PMN to evaluate the association of aPLA2R with disease activity. Antibody levels were monitored quarterly, and the authors concluded that persistence of aPLA2R was an independent risk factor for nonremission, whereas a decrease was associated with reduction in proteinuria. However, the knowledge regarding the closeness of the temporal association between change in aPLA2R titers during therapy and the disease course is not complete. Knowledge of the optimal frequency of monitoring would help in planning of individualized tailoring of immunosuppressive therapy. We examined this relationship in a cohort of patients with PMN and elevated aPLA2R by monitoring aPLA2R level at monthly intervals.

MATERIALS AND METHODS

Study Population

This prospective study was undertaken at the Postgraduate Institute of Medical Education and Research,

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Chandigarh, India. Consecutive patients who had PMN and persistent nephrotic state despite 6 months of optimal blockade of the angiotensin pathway and were treated with immunosuppressive therapy were included. Patients with diabetes mellitus, hepatitis B or C, HIV-I/II infection, or past immunosuppressive use were excluded. The Institute Ethics Committee of the Postgraduate Institute of Medical Education & Research, Chandigarh, India, approved the study, and all patients provided written informed consent.

Follow-up and aPLA2R Detection

Patients were followed up at monthly intervals, and clinical details including proteinuria, serum albumin, and serum creatinine were recorded. Estimated glomerular filtration rate was calculated using the Modification of Diet in Renal Disease (MDRD) Study equation. Serum samples were collected before starting immunosuppressive treatment and at monthly intervals and were stored at −80°C until testing. Antibody testing was done by enzyme-linked immunosorbent assay (ELISA; EUROIMMUN AG, Lubeck, Germany) according to the manufacturer's instructions, and titers ≥ 20 RU/ml were considered positive.

Definitions

Nephrotic syndrome was defined as proteinuria of >3.5 g/d or ≥2.0 g/d, along with serum albumin <2.5g/dl.[7] Complete remission was defined as proteinuria <500 mg/d with serum albumin ≥3.5 g/dl and serum creatinine, and partial remission was defined as proteinuria of 0.5 to 3.5 g/d or a <50% decline from baseline with serum albumin ≥3.5 g/dl and stable serum creatinine.[7] Serological remission was defined as aPLA2R titers of <20 RU/ml in at least 2 sequential samples. We defined the clinico-serological correlation as achievement of clinical remission (complete or partial remission) within a 3-month period following aPLA2R becoming negative.

Statistical Analysis

Data are expressed as continuous variables, percentages, means and SDs, or medians and interquartile ranges. The Student t test was used to compare means for parametric data, and nonparametric data were analyzed using the Mann−Whitney test. The χ^2 or Fisher exact test examined the association between aPLA2R positivity at the end of 6 and 9 months, respectively. Correlation between the baseline aPLA2R antibody titers, proteinuria, and serum albumin was examined by regression analysis. A P value of < 0.05 was considered significant. Statistical analysis was performed using GraphPad Prism (version 7.0; GraphPad Software, La Jolla, CA).

RESULTS

Of a total of 40 patients with IMN and persistent nephrotic syndrome seen during this period, 30 had aPLA2R-related PMN (9 female and 21 male patients). All 30 patients had persistent nephrotic syndrome after 11.9 ± 4.4 (range, 6−24) months of optimal angiotensin pathway blockade. The age of the patients was 33.2 ± 10 (range, 13−52) years. The mean proteinuria, serum albumin, and eGFR were 6.73 ± 3.93 (range, 2.43−17.20) g/d, 2.05 ± 0.63 (1.18−3.30) g/dl, and 99.3 ± 33.8 (17.4−149.9) ml/min per 1.73 m^2, respectively. Three patients had an eGFR < 60 ml/min per 1.73 m^2. Serum aPLA2R was positive at baseline in 28 patients (93.33%), and 2 patients (6.67%) showed a positive serology result in the 1-month sample. The median aPLA2R titer at baseline was 238.85 ± 300.56 (23.65−1568.35) RU/ml. Baseline parameters are provided in Table 1. There was a correlation between baseline aPLA2R and proteinuria ($r^2 = 0.56$, $P = 0.001$) but not with serum albumin ($r^2 = -0.04$, $P = 0.81$). A total of 28 patients (93.33%) received cyclical therapy with cyclophosphamide and steroids (cCTX/GC; i.v. methylprednisolone 1 g/d for 3 consecutive days, followed by oral prednisolone 0.5 mg/kg per day for 27 days in the first, third, and fifth months, and oral cyclophosphamide at 2 mg/kg per day in the second, fourth, and sixth months), and 2 patients (6.67%) were treated with rituximab (375 mg/m^2, followed by doses titrated to CD 19 counts).

Patients were followed up for 9 to 15 months. Details of individual patients are provided in Supplemental Table S1. A total of 24 patients (95.83%) had met the definition of serological remission at 6 months. Of the patients, 20 (66.7%) had achieved clinical remission at

Table 1. Baseline parameters of study patients

Parameter	Value
Age, yr	33.2 ± 10 (13–52)
Sex, male:female	21:07
Duration, mo[a]	11.9 ± 4.4 (6–24)
Proteinuria, g/d	6.73 ± 3.93 (2.43–17.20)
Serum albumin, g/dl	2.05 ± 0.63 (1.18–3.30)
Estimated GFR, ml/min per 1.73 m^2	99.3 ± 33.8 (17.4–149.9)[b]
aPLA2R (RU/ml)[c]	238.85 ± 300.56 (23.65–1568.35) Median 163.41 (IQR 70–291.01)

[a]Months of angiotensin-converting enzyme inhibitors or angiotensin receptor blockers received.
[b]Three patients had eGFR of < 60 ml/min per 1.73 m^2.
[c]Two patients had aPLA2R during follow-up.
Data are mean with range in parentheses unless otherwise indicated. aPLA2R, autoantibodies to M-type phospholipase A2 receptor; GFR, glomerular filtration rate; IQR, interquartile range.