

Original Paper

Immunohistochemical Glomerular Expression of Phospholipase A2 Receptor in Primary and Secondary Membranous Nephropathy: A Retrospective Study in an Indian Cohort with Clinicopathological Correlations

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Keywords

Immunohistochemistry · Membranous nephropathy · Phospholipase A2 receptor · Lupus nephritis

Abstract

Background: Limited published literature exists on the utility and standardization of anti-phospholipase A2 receptor (anti-PLA2R) immunohistochemistry (IHC) for the diagnosis of primary membranous nephropathy (MN). The study aimed to validate anti-PLA2R IHC for the diagnosis of primary MN and clinicopathological correlations in an Indian cohort. **Methods:** Subjects included patients with primary and secondary MN diagnosed between January 2012 and August 2014 with an adequate renal biopsy and at least 1 year of clinical follow-up. Anti-PLA2R IHC was performed in all cases with miscellaneous renal lesions as controls. Electron microscopy was performed in selected cases. Sensitivity and specificity of anti-PLA2R IHC to identify primary MN was evaluated. Histopathological analyses of primary and secondary MN were done with clinicopathological correlations including serum creatinine, eGFR, chronic kidney disease stage, 24-h urine protein, serum cholesterol, serum albumin, and hypertension at presentation and follow-up, using the Kruskal-Wallis test and Spearman rank correlation. A *p* value of ≤ 0.05 was considered statistically significant. **Results:** In 153 MN patients (99 primary, 54 secondary) and 37 miscellaneous controls, anti-PLA2R IHC differentiated primary from secondary MN with a sensitivity of 70.2% and a specificity of 96.6%. Secondary MN had increased mesangial matrix expansion compared to primary MN ($p = 0.001$). Severe nephrotic syndrome, impaired renal function, and hypertension were all more common in primary than in secondary MN. **Conclusion:** Anti-PLA2R IHC is a specific marker to distinguish primary MN from secondary MN.

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Introduction

Membranous nephropathy (MN) has a worldwide incidence of 20–30% of adult-onset nephrotic syndrome [1, 2] and 14% of cases were referred to our institute at the Christian Medical College, Vellore, India. It occurs in the 3rd–5th decade with a male-to-female ratio of 2:1. The diagnosis of this type of glomerulonephritis requires the detection of diffuse uniform capillary wall thickening by light microscopy and the visualization of subepithelial [3] immune deposits on electron microscopy (EM), resulting in a diffuse granular contiguous pattern of deposits of IgG and C3 on immunofluorescence (IF). Differentiation of primary (previously known as idiopathic MN) from secondary MN is of utmost importance for subsequent clinical management [4]. The various causes of secondary MN include systemic lupus, hepatitis B, malignancy and sarcoidosis [5, 6].

In 2009, phospholipase A2 receptor (PLA2R) was detected as the major target antigen in the pathogenesis of primary MN [7]. PLA2R is a type I transmembrane protein present on normal podocytes; there is enhanced expression of PLA2R and subsequent development of serum anti-PLA2R IgG4 autoantibodies in 70% [8] of patients with primary MN. Absence or reduction of serum anti-PLA2R antibodies is observed on immunosuppression with a low sensitivity of 57%, whereas PLA2R positivity in renal biopsy in primary MN is a more precise diagnostic tool [9]. Detection of PLA2R in renal biopsies by IF and immunohistochemistry (IHC) to distinguish primary from secondary MN has been reported with varying sensitivity and specificity. A recent study [10] showed comparable sensitivity between ELISA (74.5%), indirect IF (IIF) (72.3%), and IHC (76.6%). To our knowledge, to date only 2 studies [6, 10] have validated PLA2R IHC.

Studies [11–13] have shown that the histological assessment of tubular atrophy and interstitial fibrosis are important for the prediction of renal survival. Other independent factors [11] such as age, hypertension, and initial creatinine clearance are also important for the prognostication of primary MN. The utility of PLA2R positivity in MN has not been studied as a prognostic predictor of end-stage renal disease. There is limited published literature on the diagnostic utility of PLA2R IHC, hence this study serves to validate PLA2R IHC in an Indian cohort, highlighting its value as a diagnostic tool.

Subjects and Methods

Ethical clearance was obtained and the study was approved by the institutional review board of our institution.

Cases of MN reported from January 2012 to August 2014 were included if there was an adequate renal biopsy of ≥ 8 glomeruli with hematoxylin and eosin, periodic acid Schiff, and Jones methenamine silver stains and corresponding IF reports. To confirm the pathological evaluation, all biopsies were reviewed by 2 pathologists who are among the authors of this report. EM was done in 14 discrepant cases in whom light microscopy and IF did not provide a definitive diagnosis of MN. All cases were stained with anti-PLA2R antibody (Sigma Aldrich) using IHC on paraffin sections (online suppl. annexure; for all online suppl. material, see www.karger.com/doi/10.1159/000453675). Primary MN was defined by a clinical evaluation identifying no secondary cause, including no clinical evidence of lupus, chronic infection, malignancy or relevant drug exposure, along with negative serological testing for ANA, ANCA, HCV, HBV, and HIV. Cases were excluded if there was an incomplete evaluation for secondary etiologies or a follow-up of less than 1 year. Children and cases of allograft MN were also excluded. Data collected included age, sex, hypertension, 24-h urine protein, serum creatinine, eGFR, serum albumin, and serum cholesterol at presentation and at the last follow-up.

Table 1. Clinical characteristics of the patients with primary and secondary MN

Variables	Primary MN	Secondary MN	p values
Number of patients	99	54	–
Mean age ± SD, years	41±13.5	54±9.4	–
Gender (m/f)	73/26	4/50	–
Baseline serum creatinine, mg/dL	1.01 (0.6–10.7)	0.9 (0.5–3)	0.001
Follow-up serum creatinine, mg/dL	1.03 (0.4–5.2)	0.7 (0.4–2.6)	0.001
Baseline eGFR, ml/min/1.732 m ²	80.9 (3.9–193.9)	80.9 (26.6–218.4)	0.734
Follow-up eGFR, ml/min/1.732 m ²	79.9 (12.5–289.9)	103.7 (2.6–202.6)	0.06
Baseline 24-h urine protein, mg	5,200 (520–27,400)	1,900 (101–12,800)	<0.001
Follow-up 24-h urine protein, mg	251 (4–16,900)	135 (4–4800)	0.01
Serum cholesterol, mg	293 (224,814)	224 (87,506)	0.007
Serum albumin, mg	2.2 (1.2–4.9)	3.1 (1–4.5)	<0.001
Hypertension (yes/no)	19/77 (24.7%)	1/49 (2%)	0.003

Figures are medians with ranges in parentheses, unless indicated otherwise.

Statistical Methods

For the description of data, we used mean with standard deviation or median with range for continuous data, and frequency along with percentage for categorical data. The diagnostic accuracy for IHC is given with a 95% confidence interval and the Kruskal-Wallis test. A p value of ≤0.05 was considered statistically significant.

Results

A total of 153 subjects with MN diagnosed between January 2012 and August 2014 were included in the study. All had adequate renal biopsies and clinical data for at least 1 year of follow-up. There were 76 males and 77 females, aged 18–73 years. The cohort included 99 with primary MN and 54 with secondary MN. All cases of secondary MN were due to class V lupus nephritis. In addition, 37 miscellaneous renal biopsies with conditions not associated with PLA2R positivity [5–7] were studied as controls. The miscellaneous cases included focal segmental glomerulosclerosis (n = 4), IgA nephropathy (n = 9), diabetic nephropathy (n = 13), class IV lupus nephritis (n = 6), membranoproliferative glomerulonephritis (n = 3), and sarcoidosis (n = 2). EM was done in 14 cases with a discrepancy in the diagnosis. Being a tertiary care center, most of the patients in our institution were either referred or treated elsewhere with immunosuppressants.

Pathological Findings in Primary Compared to Secondary MN

The predominant stage of primary MN in our study was stage 3, 51/99 (51%) by light microscopy supported by EM in the selected cases. Few cases of stage 4 MN were detected by EM in our cohort. Mixed stages of stage 1–2 and stage 2–3 comprised 5/99 (5%) and 15/99 (15%), respectively. Systemic lupus erythematosus (class V lupus nephritis) was the leading cause of secondary MN in all cases in this cohort.

Median serum creatinine at the time of biopsy was higher in primary MN (1.01 [0.57–10.7] mg/dL) than in secondary MN (0.88 [0.5–3] mg/dL) and was also higher at 1 year (primary MN 1.03 [0.37–5.2] mg/dL vs. secondary MN 0.71 [0.41–2.64] mg/dL) (Table 1). Urine protein was also higher in primary MN (5,200 mg/24 h [520–27,400]) than in secondary

Table 2. Histological assessment of mesangial matrix expansion and PLA2R IHC in primary and secondary MN

Variables	MN		
	primary	secondary	
PLA2R			
Positive	66	2	68 ^b
Negative	28	57 (52 + 5 ^a)	85 ^b
Total	94	59	153 ^b
Mesangial matrix expansion			
Mild	58/99 (58.6%)	14/54 (25.9%)	0.001 ^c
Moderate	36/99 (36.4%)	35/54 (64.8%)	
Severe	5/99 (5%)	5/54 (9.3%)	

^a Five discrepant cases confirmed by EM. ^b Total number. ^c *p* value.

MN (1,900 mg/24 h [101–12,800]) at the time of biopsy as well as at 1 year (primary MN 251 mg/24 h [4–16,900] vs. secondary MN 135 mg/24 h [4–4,800]).

Serum cholesterol levels were higher in primary MN (293 mg/dL [224–814]) compared to secondary MN (224 mg/dL [87–506]). Serum albumin was lower in primary MN (2.2 mg/dL [1.2–4.9]) compared to secondary MN (3.1 mg [1–4.5]). Hypertension was more common in primary MN (24.7%) compared to secondary MN (2%).

Histopathological comparison of glomerular, tubulointerstitial and vascular compartments of all the cases showed significantly increased mesangial matrix expansion in secondary compared to primary MN ($p < 0.001$) (Table 2).

PLA2R Immunohistochemical Analysis

Positive immunohistochemical staining of PLA2R was defined by the complete positive staining of the glomerular capillary walls (Fig. 1a). Negative controls had isolated faint granular peripheral staining of the podocytes as described in the literature [6] (Fig. 1b, c). Immunohistochemical staining ranged from weak positivity (1+) to strong positivity (3+).

Analysis of Intensity of Anti-PLA2R IHC

PLA2R IHC was positive in 66/99 cases of primary MN and in 2/54 cases of secondary MN (Table 2). A total of 21 cases showed 3+, 32 cases showed 2+ and 13 cases showed 1+ positivity. Two cases of suspected systemic lupus erythematosus showed 1+ positivity. None of the 37 miscellaneous controls showed positivity.

PLA2R IHC had a sensitivity of 70.2% (95% CI 65.2–72.2) and a specificity of 96.6% (95% CI 88.3–99.6) to differentiate primary MN from secondary MN. Five patients initially identified as primary MN had negative PLA2R staining but further analysis by EM showed mesangial deposits in all cases and subendothelial deposits in 2 of the cases (Fig. 2a–c) suggestive of a secondary etiology. One patient proved to have membranous lupus nephritis and in a second case further clinical evaluation revealed a concurrent papillary renal cell carcinoma leading to a diagnosis of malignancy-associated secondary MN. Follow-up evaluation of all the other cases identified as primary MN did not reveal any cause for secondary MN.

Clinicopathological Analysis of PLA2R

PLA2R positivity was analyzed with respect to the association with the renal function status in patients with primary MN and it was detected that there was no significant association between PLA2R positivity and the rate of decline in renal function at the end of the last follow-up of a minimum of 1 year ($p = 0.96$).

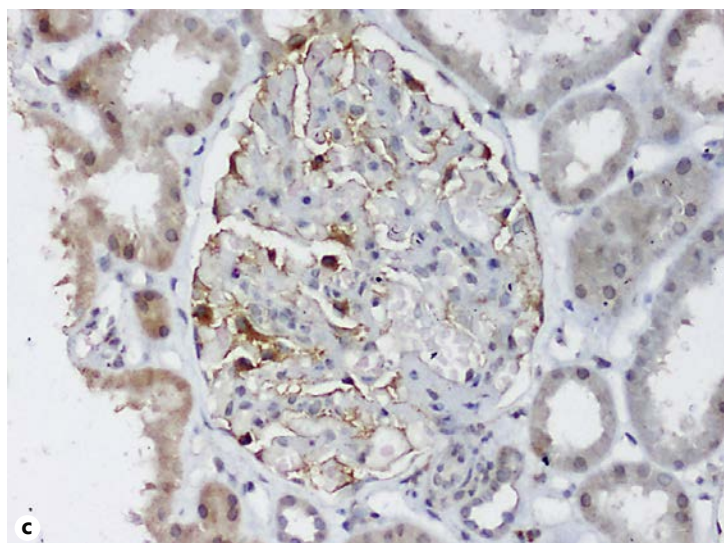
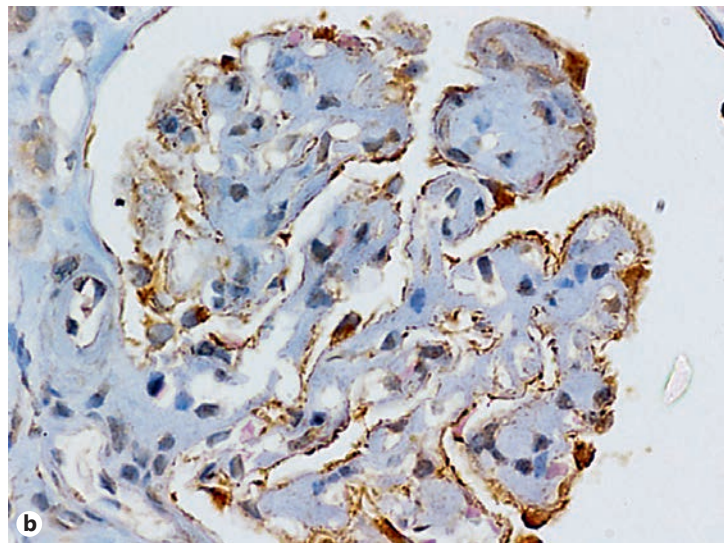
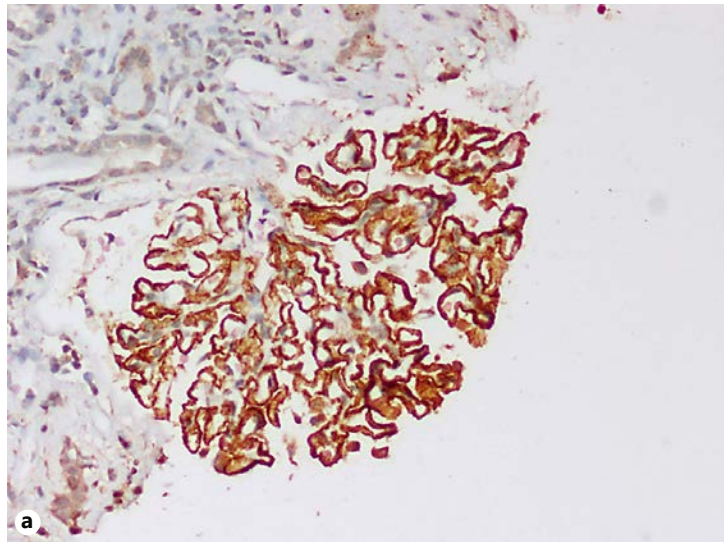


Fig. 1. a–c PLA2R immunohistochemistry ($\times 200$): PLA2R (3+) positive staining (**a**) along glomerular capillary walls, PLA2R negative staining in IgA nephropathy (**b**) and systemic lupus erythematosus class V lupus nephritis (**c**).

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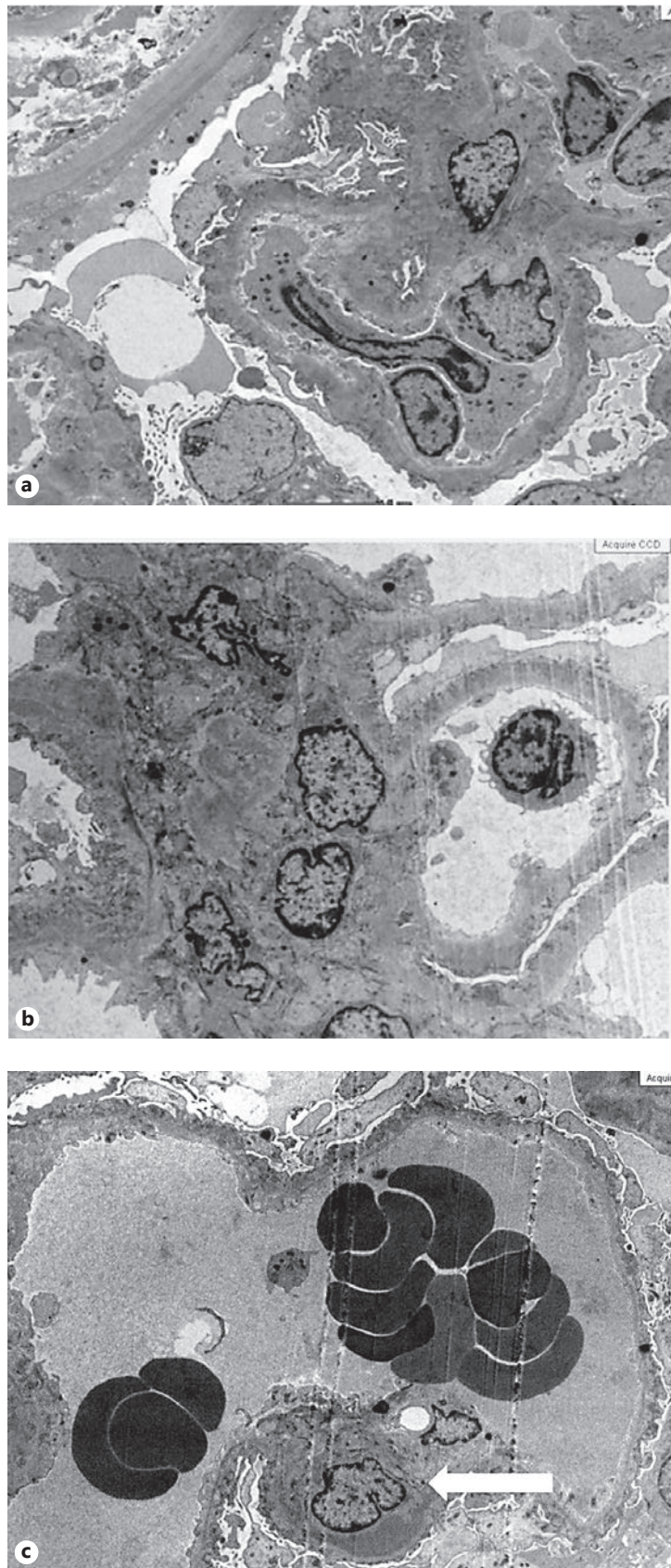


Fig. 2. a–c Electron microscopy of discrepant cases (transmission electron microscopy, $\times 2,550$). **a** Membranous nephropathy with endocapillary cell proliferation. **b** Membranous nephropathy with many mesangial electron-dense deposits and increased mesangial cellularity. **c** Subendothelial electron-dense deposit (arrow) in a case of membranous nephropathy.

Discussion

The recent detection of M-type PLA2R [7] as a target antigen in primary MN with circulating anti-PLA2R antibodies correlating with disease activity has greatly influenced the management and the distinction of primary from secondary MN and particularly, lupus nephritis [9].

It has been observed that the corresponding PLA2R antibodies to the antigens were not thoroughly reliable and were particularly variable in an individual. Their detection depended on various factors such as previous treatment history, time of detection of antibodies, and remission rates, with the values occasionally not coinciding with the disease at present [6, 8], whereas PLA2R antigen detection in a renal biopsy was almost persistent in primary MN [9]. Considering the fact that anti-PLA2R antibodies have been shown to be inconsistent with regard to their rapid clearance from circulation and their false positivity in a few patients with secondary MN, the diagnosis of primary MN based on the detection of anti-PLA2R antibodies is a major hindrance. Thus, PLA2R antigen detection in renal biopsy is an essential step that is required in the diagnosis of primary MN.

The cost of detection of serum anti-PLA2R antibodies is 5 times higher than that of IHC in a renal biopsy for the diagnosis of primary MN, precluding its routine use as a diagnostic test.

Furthermore a negative PLA2R staining by IHC has been reported by Beck et al. [7] to show positivity of only the podocytes at the glomerular peripheral capillary walls. This feature provides an optimum internal control for IHC that is not detectable on IIF of paraffin sections. Hence, our study aimed to optimally validate the diagnostic usefulness of anti-PLA2R IHC for the diagnosis of primary MN.

The detection of PLA2R antigen has been carried out by IIF, Western blot and ELISA with limited reports on immunohistochemical analysis.

A comparative study from Spain [10] showed that the sensitivity of PLA2R IHC was 76.6%, comparable to 72.3% using IIF and 74.5% using ELISA. PLA2R IHC has been found to have a sensitivity ranging from 84% [6] to 77% [10], with a specificity of 100% [6] to 94% [10] for the differentiation of primary from secondary MN. Limited data exist related to its efficiency and validation as a diagnostic tool in India, which is important since IHC is widely available in Indian laboratories, highlighting the need for further studies on Indian cohorts.

We here report a retrospective study on 153 renal biopsies over 2 years, which included 99 patients with primary MN (73 males, 23 females), 54 patients with secondary MN (all class V lupus nephritis, which is the commonest cause of secondary MN at our institution), and 37 controls with miscellaneous glomerular lesions.

56% of the patients in our study group had marked glomerular capillary wall thickening and 39% had chronic kidney disease stage 1. The majority of the patients presented with adult-onset nephrotic syndrome. Our study showed a statistically significant difference between clinical features of primary and secondary MN. Severe nephrotic syndrome, impaired renal function, and hypertension were all more common in primary MN.

Our analysis of PLA2R glomerular staining by IHC showed a lower sensitivity of 70.2% and a comparable specificity of 96.6% compared to 2 previous studies using IHC and IIF (Table 3). The relatively lower sensitivity for PLA2R in our study might be explained by an underlying etiology for secondary MN, which was not detected in our initial clinical and laboratory evaluation. No such causes emerged within at least 1 year of follow-up, but this does not absolutely exclude the possibility that other causes would emerge during longer follow-up. The other possibility is that podocyte antigens other than PLA2R were the target in some cases of primary MN – for example, recently described antigens such as superoxide dismutase, aldose reductase [14, 15], and thrombospondin type 1 domain-containing 7A

Table 3. Comparison review of previous studies on PLA2R on renal biopsy

First author [Ref.], year	Total number of subjects	Assay method	Sensitivity, %	Specificity, %
Hoxha [6], 2012	88	IHC	84.0	100.0
Segarra-Medrano [10], 2014	64	IHC	76.6	94.0
Larsen [5], 2012	165	IIF	75.0	83.0
Svobodova [9], 2013	84	IIF	80.0	84.0
Barrett [17], 2014	7	IIF	67.0	100.0
Gasim [18], 2014	41	IIF	77.0	94.0
Debiec [8], 2014	42	IIF	74.0	–
Present study	153	IHC	70.2	96.6

antigen [16] that has been detected to play a similar significant role in the pathogenesis of primary MN.

A detailed light microscopic analysis of glomerular, tubulointerstitial and vascular compartments was done looking for differences between primary and secondary MN. Patients with secondary MN had a higher degree of mesangial matrix expansion compared with primary MN, in concordance with results of previous studies stating greater prevalence of mesangial proliferation in secondary MN [3]. It was also interesting to note that PLA2R positivity was not useful for prognostication in primary MN. Analysis of lesions of the tubulointerstitial compartment [11–13, 19, 20] including tubular atrophy/interstitial fibrosis continue to be the most important histological prognostic indicators.

In summary, we conclude that in Indian subjects with MN PLA2R staining of renal biopsies using IHC is a rapid, accessible, reproducible specific test and a permanent record for the diagnosis of primary MN. Mesangial matrix expansion assessment is useful for the differentiation of primary from secondary MN.

Limitations

Class V lupus nephritis formed the major cohort of secondary MN in our study as this is the predominant proven cause of secondary MN in India as opposed to other forms of secondary MN, where a concomitant primary MN cannot be excluded.

Serum anti-PLA2R estimation was not performed for patients with primary MN. The authors acknowledge that although this was considered a requirement at the time of the first diagnosis of primary MN, when considering the cost-effectiveness, sensitivity and specificity, anti-PLA2R IHC has been proven to be a superior and more reliable diagnostic tool than the detection of serum anti-PLA2R antibodies at the time of renal biopsy in our experience.

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Statement of Ethics

The patients included in the study have given their informed written consent for the study and the study protocol was approved by the institution ethics committee in accordance with the World Medical Association Declaration of Helsinki.

Disclosure Statement

The authors declare no conflicts of interest.

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