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Clinical and Histopathologic Characteristics of Pediatric Patients With Primary Membranous Nephropathy

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Introduction: Primary membranous nephropathy (PMN) is uncommon in children. Therefore, data on the clinical course of affected children are scarce. In recent years, several novel antigens have been implicated in the pathogenesis of PMN. However, the histopathologic characteristics of pediatric patients with PMN remain poorly represented in the literature.

Methods: We have retrospectively analyzed the clinical presentation and outcomes data of 21 children with PMN from 3 centers in the United States. In addition, we have identified novel antigens in biopsy specimens from these patients and correlated their presence or absence to clinical outcomes. Finally, we compared the results of the novel antigen staining from our clinical cohort to a validation cohort of 127 biopsy specimens from children with PMN at Arkana Laboratories.

Results: The data from the 2 cohorts demonstrated similar overall antigen positivity rates of 62% to 63%, with phospholipase A2 receptor (PLA2R) and exostosin 1 (EXT1) being the most commonly found antigens. Results from the clinical cohort showed that overall, the kidney prognosis for children with PMN was good, with 17 of 21 patients entering a complete or partial remission. Children who were positive for PLA2R or EXT1 were significantly more likely to enter remission than those in the antigen negative group.

Conclusion: Approximately 60% of pediatric membranous cases are positive for a novel antigen on kidney biopsy and the clinical prognosis is generally favorable. More studies are needed to understand the clinical implications of each specific novel antigen.

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Membranous nephropathy is a common cause of nephrotic syndrome in adults, of which 70% to 80% of cases are primary or idiopathic.[1] Membranous nephropathy is a histologic diagnosis characterized by immunofluorescence demonstrating immunoglobulin G (IgG) and typically complement C3 deposition along the glomerular capillary loops. PMN refers to that which is kidney-limited and immune-mediated, as opposed to secondary membranous nephropathy, which is typically due to infections, malignancy, medications, drugs, or other systemic diseases. PMN is uncommon in the pediatric population, accounting for 1% to 5% of all children with nephrotic syndrome.[2-6] PMN accounts for a larger proportion of nephrotic syndrome in steroid-resistant pediatric patients and in patients with nephrotic syndrome between the ages of 13 and 19 years.[6-10]

In recent years, novel autoantigens have been discovered that have been implicated in PMN. In 2009,

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Beck *et al*.[11] first described the PLA2R as a major target antigen in adult disease; and over the last decade, many new antigens have been noted to be present in biopsy specimens of patients with MN in largely adult cohorts. These antigens include thrombospondin type-1 domain containing 7A (THSD7A),[12] exostosins 1 and 2 (EXT1/2),[13] nerve epidermal growth factor like-1 (NELL1),[14] protocadherin 7,[15] neural cell adhesion molecule 1 (NCAM1),[16-18] semaphorin 3B (SEMA3B),[19] transforming growth factor beta receptor 3 (TGFBR3),[20] serine protease HTRA1,[21] neuron- derived neurotropic factor,[22] and protocadherin FAT1[23] in addition to other minor antigens.[24]

The current trend for the classification of PMN is for it to be based on novel antigen detection as opposed to the classic "primary" and "secondary" terminology, and it is important to have pediatric studies to further our understanding of these antigens and associated disease outcome in children. Overall, children with PMN are inadequately represented in the medical literature with only a few pediatric case series reported in the modern era.[2,4,25-30] O'Shaughnessy *et al*.[31] reported treatment patterns in children with PMN enrolled in the Cure Glomerulopathy Network, but to date, data on novel antigens and their clinical implications in pediatric PMN is scarce. We sought to gather a cohort of pediatric patients with PMN to evaluate the histopathologic characteristics, namely to identify the frequency of the presence of the previously noted antigens, as well as to correlate this with a patient's clinical course and response to treatment.

METHODS

Patient Cohorts

Patients with biopsy diagnosis of PMN between 2003 and 2021, aged 18 years or less, were retrospectively identified through existing databases of renal biopsy specimens and billing records at Indiana University, University of Minnesota, and University of Iowa. Biopsy specimens were diagnosed by brightfield, immunofluorescence, and transmission electron microscopy using conventional techniques.[32] Requisite histopathologic criteria for diagnosis of membranous nephropathy included detection of aggregated IgG and complement C3 along glomerular capillary loops, which ultrastructurally correlated to subepithelial electron dense deposits. Patients' charts were reviewed for clinical presentation, treatment, and outcomes data. Patients with secondary illness, including autoimmune diseases such as systemic lupus erythematosus, diabetes mellitus, celiac disease, hepatitis B/C, or other evidence of another concurrent illness or systemic disease were excluded. Specimens with a concurrent proliferative glomerulonephritis component were also excluded.

Biopsy specimens were stained for PLA2R, THSD7A, EXT1, NELL1, SEMA3B, NCAM1, and TGFBR3 by paraffin immunofluorescence by an independent pathologist at Arkana Laboratories. The remainder of the biopsy data are reported per the read from the local pathologist.

The histopathological results from this cohort were then compared to a larger cohort of 127 biopsy specimens of children $\leq$ 18 years of age with PMN identified at Arkana Laboratories. These cases were stained with PLA2R, THSD7A, EXT1, NELL1, SEMA3B, and NCAM1. Clinical follow-up information was not available.

Immunostaining for Membranous Nephropathy Antigens

THSD7A, EXT1, SEMA3B, NCAM1, and TGFBR3 staining was performed following antigen retrieval by heating 3 μm tissue sections at 99 °C in high-pH citrate buffer (BOND Epitope Retrieval Solution, pH 9.0, cat # PA5-21994, Invitrogen). PLA2R and NELL1 staining was performed following antigen retrieval through treatment of tissue sections with proteinase K solution for 20 minutes at room temperature. Primary antibodies used for staining the protein targets in membranous nephropathy included rabbit polyclonal anti-PLA2R (1:50 dilution, cat # HPA012657, Sigma), mouse monoclonal anti-THSD7A (1:100 dilution, cat # AMAB91234, Atlas antibodies), rabbit polyclonal anti-NELL1 (1:50 dilution, cat # PA5-27958, Invitrogen), rabbit polyclonal anti-NCAM1 (1:50 dilution, cat # HPA039835, Sigma), rabbit polyclonal anti-EXT1 (1:50 dilution, cat # PA5-60699, Invitrogen), rabbit polyclonal anti-TGFBR3 (1:25 dilution, cat # HPA008257, Sigma), mouse monoclonal HTRA1/PRSS1 antibody (1:50 dilution, cat # MAB2916, R&D systems), and rabbit polyclonal anti-SEMA3B (1:200 dilution, cat # ab48197, AbCam). Primary antibodies were incubated for 40 minutes at room temperature, followed by washing in phosphate-buffered saline and incubation with secondary antibodies. Secondary antibodies included Rhodamine Red X-conjugated goat anti-rabbit IgG (1:100 dilution, cat # 111-295-144, Jackson ImmunoResearch) or FITC-conjugated goat anti-mouse IgG (1:100 dilution, cat # 115-095-207, Jackson ImmunoResearch), dependent on the species of the primary antibodies, and were reacted with tissue sections for 40 minutes at room temperature. Sections were coverslipped in aqueous mounting medium and examined by immunofluorescence microscopy. Staining was considered to be positive if there was 2+ or greater granular capillary loop immunoreaction within

glomeruli, and negative if there was no capillary loop signal within glomeruli (intensity scale from 1+ to 4+).

Clinical and Laboratory Data

Within the study, the following definitions were used:

- Estimated glomerular filtration rate was calculated using the bedside Schwartz equation.[33]
- Hypoalbuminemia was defined as a serum albumin less than 3.0 mg/dl
- Proteinuria at presentation was defined as any urine protein-to-creatinine ratio (UPCR) above 0.2 mg/mg.
- Complete remission was defined as a UPCR of less than 0.3 mg/mg.[34]
- Partial remission was defined as a 50% reduction in proteinuria from presentation.[34]
- Hematuria was defined as greater than 5 red blood cells per high power field.

Statistical Analysis

Study data were collected and managed using REDCap electronic data capture tools hosted at Indiana University.[35,36] Studies were approved by each center's institutional review board.

Comparison groups included immunosuppression treatment yes/no, remission status yes/no, and antigen PLA2R/EXT1/negative. Categorical measures are summarized as rates and compared between groups using Fisher exact test or Kruskal-Wallis rank test. Continuous measures are summarized as medians with first and third quartiles and compared between groups using the Wilcoxon rank-sum test. Longitudinally collected measures, including serum albumin, UPCR, and estimated glomerular filtration rate were analyzed over time and compared between groups using linear mixed-effects models with fixed effects for time, group, and time-by-group interaction, and a random effect for patient to account for within-patient correlation. Mixed-effects models provide unbiased estimates of mean responses in the presence of missing observations/loss to follow-up. Similar models that included a fixed effect term for the baseline (presentation) measurement were fit to obtain tests for group differences that are adjusted for baseline values. Time to remission was summarized using the Kaplan-Meier estimator and compared by immunosuppression status using the log-rank test. Analyses were conducted using R version 4.0.1 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Clinical Cohort

Of 29 patients identified with membranous nephropathy on biopsy at Indiana University, University of Iowa, and University of Minnesota, 21 patients had biopsy tissue for further evaluation. The biopsy reports were reviewed and data reported herein. The demographic and clinical characteristics of the clinical cohort at presentation can be found in Table 1. Pediatric patients with PMN were predominantly female (12/21, 57%) with a median age of 13 years. The majority of patients presented with nephrotic range proteinuria and with preserved kidney function. The median time from presentation to biopsy was l0 days.

Of the 21 children, 13 (62%) were positive for PLA2R ($n = 8$) or EXT1 ($n = 5$) (Table 1). In Table 2, we describe the histologic patterns in detail for 13 of 21 patients for which biopsy reports were available. One patient was identified as having "full house" staining on immunofluorescence, but did not have any other evidence of systemic lupus erythematosus. This patient was also EXT-1 negative. In addition, none of the EXT-1 positive patients had C1q positivity on immunofluorescence. Based on our limited data, there were no discrete immunofluorescence patterns, percent global or segmental sclerosis, or interstitial fibrosis and tubular atrophy identified that consistently appeared in any group of patients.

Treatment and Follow-Up

There were 18 patients with treatment data available, of which 11 (61%) were treated with immunosuppression. Nine (81%) patients were treated with prednisone, 1 (9%) with mycophenolate mofetil, and 1 (18%) with rituximab. Prednisone was used as a first line therapy for all the patients with immunosuppressive treatment with the exception of 1 patient who received rituximab as initial treatment. There was no statistically significant difference in the number of patients treated with immunosuppression between the antigen groups. However, patients who were treated with immunosuppression had a lower median serum albumin at presentation as compared to those who were treated with conservative therapy (2.5 mg/dl vs. 2 mg/dl, $P = 0.019$). The UPCR in the treatment group tended to be higher at presentation in patients treated with immunosuppression (median 6 mg/mg vs. 2.8 mg/mg, $P = 0.066$).

The median follow-up time was 2.18 years (interquartile range 1.36, 3.44). All children with available follow-up data (17 patients) entered either a complete or partial remission. Patients with PLA2R or EXT1 positivity tended to have a lower UPCR at the most-recent follow-up compared to the antigen negative group ($P = 0.094$). All 5 EXT1 patients entered a complete remission; 2 of the EXT1+ patients were treated with prednisone, whereas 3 were treated conservatively without immunosuppression.

Table 1. Clinical characteristics and outcomes

		Age (yrs)		Antigen				
		3–11	12+	PLA2R	EXT1	NEG		
Characteristics	***N* = 21**	***n* = 6**	***n* = 15**	***n* = 8**	***n* = 5**	***n* = 8**	***P*-value**	**% missing**
Sex (% female)	12 (57)	3 (50)	9 (60)	4 (50)	3 (60)	5 (63)	0.762	0
Age (yrs) (median [IQR])	13 [10, 15]	8.00 [5.25, 10.00]	14.00 [13.00, 16.40]	13.5 [13,15]	14 [12,14]	11.5 [9,17]	0.851	0
BMI (kg/m^2) (median [IQR])	22 [20.1, 33.8]	18.10 [17.65, 18.65]	27.20 [21.80, 33.80]	21.7 [20.6, 26.6]	29.3 [26.5, 35.6]	19.4 [18.4, 33.8]	0.288	19
Race (% Black or African American)	5 (24)	1 (17)	4 (27)	2 (25)	2 (40)	1 (12.5)	0.604	0
Ethnicity (% Hispanic or Latino)	1 (10)	1 (50.0)	0 (0.0)	0 (0)	0 (0)	1 (33.3)	0.274	52.4
Hematuria at presentation (%)	6 (33)	2 (40)	4 (31)	3 (43)	1 (25)	2 (29)	0.690	14.3
UPCR at presentation (median [IQR])	4.80 [3.00, 8.60]	6.00 [4.50, 8.71]	4.80 [2.90, 8.60]	4.80 [3.75,10.15]	5.48 [3.67,8.93]	4.90 [3.38,5.28]	0.960	19
Nephrotic Range Proteinuria at presentation (%)	15 (88.2)	4/4 (100)	11/15 (84.6)	6 (85.7)	4 (100)	5 (83.3)	0.699	19
Serum albumin at presentation (gm/dl) (median [IQR])	2.3 [2.0, 2.5]	1.80 [1.00, 2.72]	2.30 [2.00, 2.40]	2.3 [2.0, 2.5]	2.3 [1.8, 2.4]	2.3 [2.1, 2.9]	0.941	19
Nephrotic Syndrome at Presentation (%)	15 (71)	4 (66.7)	11 (73.3)	6 (85.7)	4 (100)	5 (83.3)	0.699	19
Days from presentation to biopsy (median [IQR])	13 [4, 45]	37 [9.75, 69]	8.00 [3.00, 34.00]	5.5 [0.8,14]	27 [8,31]	33 [11, 47]	0.345	0
eGFR at presentation (ml/min/1.73 m^2)	111 [96, 133]	128 [122, 133]	104 [89, 124]	105 [94,115]	104 [98,138]	133 [124,134]	0.539	28.6
Treatment with immunosuppression (% yes)	11/18 (61)	5 (83)	4 (33)					14.3
Prednisone	9 (50)	5 (83)	4 (33)	3 (43)	2 (40)	4 (67)		14.3
Prednisone + MMF	1 (6)	0 (0.0)	1 (8.3)	1 (14)	0 (0)	0 (0)		
Rituximab	1 (6)	0 (0.0)	1 (8.3)	1 (14)	0 (0)	0 (0)		
Serum albumin at 1 yr (mg/dl) (median [IQR])	4.0 [3.5, 4.3]	3.70 [3.40, 3.70]	4.20 [3.50, 4.40]	4.2 [3.7, 4.2]	4.1 [3.7,4.4]	3.4 [3.2,4.0]	0.131	42.9
UPCR at 1 yr (mg/mg) (median [IQR])	0.17 [0.08, 0.42]	0.09 [0.07, 0.98]	0.21 [0.08, 0.58]	0.19 [0.09, 0.58]	0.08 [0.07, 0.53]	0.58 [0.39, 1.23]	0.348	42.9
Serum albumin at most recent follow-up (mg/dl) (median [IQR])	4.0 [3.6, 4.3]	3.65 [1.83, 4.12]	3.95 [3.60, 4.32]	3.8 [3.6, 4.4]	4.1 [3.9,4.3]	3.8 [3.4, 4.2]	0.109	14.3
UPCR at most recent follow-up (mg/mg) (median [IQR])	0.17 [0.08, 1.42]	0.56 [0.44, 0.73]	0.70 [0.66, 0.84]	0.22 [0.12, 1.29]	0.08 [0.08, 0.12]	0.90 [0.10, 2.11]	0.094	14.3
% Remission							0.075	19
Complete	11 (61)	4 (67)	7 (58)	4 (57)	5 (100)	2 (33.3)		
Partial	7 (39)	2 (33)	5 (42)	3 (43)	0 (0)	4 (66.7)		
eGFR at most recent follow-up (ml/min per 1.73 m^2)	106 [84, 122]	131 [110, 148]	99 [84, 109]	108 [91,110]	95 [82,99]	125 [110,138]	0.222	19

eGFR, estimated glomerular filtration rate; EXT1, exostosin 1; IQR, interquartile range; MMF, mycophenolate mofetil; NEG, antigen negative; PLA2R, phospholipase A2 receptor; UPCR, urine protein-to-creatinine ratio.

Table 2. Pathologic characteristics of children with idiopathic membranous nephropathy

	LM			IF						EM			
Specimen	% GS	% SS	IFTA	+/− C3	+/− IgA	+/− IgG	+/− IgM	+/− C1q	+/− antigen	SP	MES	SN	MN Stage
1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	1+PLA2R	n/a	n/a	n/a	n/a
2	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	-	+	+	-	III
3	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	-	n/a	n/a	n/a	n/a
4	0	0	Minor	2+	1–2+	4+	2+, MES	-	3 + EXT1	+	-	-	II/III
6	0	0	0	3–4+, CL, MES	-	2+, CL, MES	-	-	-	+	-	-	II/III
7	0	0	0	4+	1+	4+	-	-	3+ EXT1	+	+	-	II
8	0	0	10%	4+	-	4+	-	-	1+ PLA2R	+	-	-	III
9	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	2-3+ PLA2R	n/a	n/a	n/a	n/a
10	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	-	n/a	n/a	n/a	n/a
13 (a)	0	0	0	1+	1+	3+	tr, MES	tr	n/a	+	+	-	III
13 (b)	0	0	0	-	tr	1+	1+, MES	-	-	+	+	+	IV
16	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	3+ PLA2R	n/a	n/a	n/a	n/a
17	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	3+ PLA2R	n/a	n/a	n/a	n/a
18	0	0	0	1+	1+	4+	1+, MES	-	-	+	+	+	III
19	0	0	0	1-2+	-	4+	1+, MES	-	3+ PLA2R	+	-	-	III
20	n/a	n/a	n/a	1+, CL, MES	-	2+	-	-	-	+	+	-	I
21	n/a	n/a	n/a	-	-	3+	-	-	2+ EXT1	n/a	n/a	n/a	n/a
22	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	2+ PLA2R	n/a	n/a	n/a	n/a
23	0	1/40	0	1+, MES	-	-	1+, MES	-	-	n/a	n/a	n/a	n/a
25	0	0	0	+	-	2-3+	-	-	+ EXT1	+	+	+	I/II
26	0	0	0	1+	-	2-3+	-	-	+ EXT1	+	-	-	III/IV
29	0	0	10	1+	-	3+	-	-	+ PLA2R	+	-	-	II

CL, capillary loops; GS, global sclerosis; IFTA, interstitial fibrosis and tubular atrophy; MES, mesangium; n/a, not applicable/information not available; SE, subendothelial; SS, segmental sclerosis; SP, subepithelial; tr, trace.

There was no statistically significant difference in complete remission rates between the immunosuppression and non-immunosuppression groups, the time to remission (Figure 1), or estimated glomerular filtration rate at follow-up. No patient required dialysis or kidney transplant.

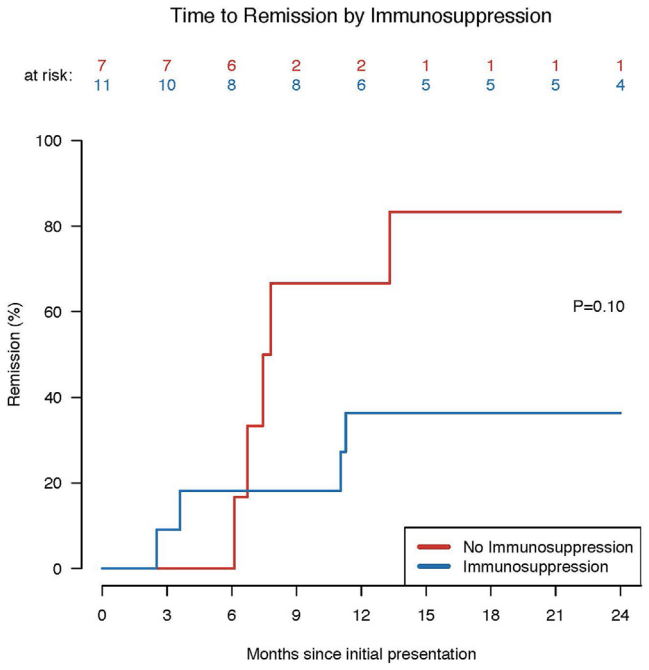

Figure 1. Kaplan Meier Curve illustrating time to remission for patients with immunosuppression (blue) and without immunosuppression (red).

Antigen Distribution of Pediatric PMN from a Larger Validation Cohort

Pediatric patients with PMN were predominantly adolescents with a median age of 15 years, showed a female predominance (59%), and had preserved kidney function (serum Cr 0.6 mg/dl of those with available data). Of the 127 patients, 41 were PLA2R positive (32%), 1 was THSD7A positive (0.8%), 28 were EXT1/2 positive (22%), 6 were SEMA3B positive (4.7%), 4 were NELL1 positive (3.1%), 1 was HTRA1 positive (0.8%), and 1 was NCAM1 positive (0.8%). The remaining 45 cases (36%) were negative for each of the 7 antigen types. Histopathologic assessment varied in the validation cohort from the initial cohort in that HTRA1 staining was assessed and TGFBR3 staining was not performed. See Tables 3 and 4 for a comparison of

Table 3. Comparison of antigen positivity between the clinical cohort and the larger validation cohort

Antigens	Clinical cohort *N* = 21	Validation cohort *N* = 127
PLA2R	8 (38)	41 (32)
EXT1/2	5 (24)	28 (22)
SEMA3B	0	6 (4.7)
NELL1	0	4 (3.1)
THSD7A	0	1 (0.8)
NCAM1	0	1 (0.8)
Antigen negative	8 (38)	46 (36)

EXT, exostosin 1; NCAM1, neural cell adhesion molecule 1; NELL1, nerve epidermal growth factor like-1; PLA2R, phospholipase A2 receptor; SEMA3B, semaphorin 3B; THSD7A, thrombospondin type-1 domain containing 7A.

Table 4. Biopsy characteristics by antigen (validation cohort)

Antigen	*n*	IgA	IgG	IgM	C3	C1q	Full house	TBM deposits	Tissue ANA	Mesangial deposits
PLA2R	41	9/41 (22.0%)	41/41 (100%)	8/41 (19.5%)	35/41 (85.4%)	1/41 (2.4%)	0/41 (0%)	0/41 (0%)	0/41 (0%)	17/41 (41.5%)
THSD7A	1	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
EXT1/2	28	13/28 (46.4%)	28/28 (100%)	10/28 (35.7%)	23/28 (82.1%)	6/28 (21.4%)	5/28 (17.9%)	7/28 (25%)	8/28 (28.6%)	25/28 (89.3%)
NELL1	4	3/4 (75%)	4/4 (100%)	3/4 (75%)	3/4 (75%)	0/4 (0%)	0/4 (0%)	1/4 (25%)	0/4 (0%)	2/4 (50%)
SEMA3B	6	2/6 (33%)	6/6 (100%)	3/6 (50%)	5/6 (83.3%)	2/6 (33%)	0/6 (0%)	0/6 (0%)	0/6 (0%)	5/6 (83.3%)
HTRA1	1	0/1 (0%)	1/1 (100%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
NCAM1	1	1/1 (100%)	1/1 (100%)	1/1 (100%)	1/1 (100%)	1/1 (100%)	1/1 (100%)	1/1 (100%)	0/1 (0%)	1/1 (100%)
Unknown	45	10/45 (22.2%)	45/45 (100%)	13/45 (28.9%)	28/45 (62.2%)	9/45 (20%)	3/45 (6.7%)	2/45 (4.4%)	2/45 (4.4%)	32/45 (71.1%)

ANA, anti-nuclear antigen; C3, complement C3; EXT1/2, exostosins 1/2; HTRA1, serine protease HTRA1; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; NCAM1, neural cell adhesion molecule 1; NELL1, nerve epidermal growth factor like-1; PLA2R, phospholipase A2 receptor; SEMA3B, semaphorin 3B; THSD7A, thrombospondin type-1 domain containing 7A; TBM, tubular basement membrane.

the antigen distribution between the clinical cohort and the larger validation cohort.

Histopathology revealed that the majority of cases demonstrated intact kidney parenchyma with a mean global glomerulosclerosis of only 4.4 ± 10.0%. Interstitial fibrosis and tubular atrophy were absent in 95 (75%), mild in 25 (20%), moderate in 5 (3.9%), and severe in 2 (1.6%) patients. Immunofluorescence was reported to show 1+ or greater IgA deposition in 38 (30%), IgG in 127 (100%), IgM in 38 (30%), C3 in 96 (76%), and C1q in 18 (14%). "Full house" immunofluorescence was seen in 11 cases (8.7%). Ten cases were reported to show "tissue ANA" staining (7.9%). Eleven cases showed tubular basement membrane deposits (8.7%). Ultrastructural evaluation revealed subepithelial electron-dense deposits in all cases, mesangial deposits in 82 (65%) cases, with the majority of cases demonstrating severe podocyte foot process effacement (84%).

DISCUSSION

This study shows that a significant portion of children with PMN are positive for novel antigens on kidney biopsy, albeit at a slightly different proportion than what has been previously described in the literature. In our cohorts, EXT1/2 positive cases were more prevalent than in adult cohorts, with a lower rate of PLA2R positivity.[37] When compared to another pediatric study by Miller *et al.*,[30] our patients had a larger percentage of antigen positivity (46% vs. 62%–63% in our study). In addition, our study had a lower frequency of SEMA3B+ patients in PMN (4.7%) and had a higher mean age than the SEMA3B+ cohort by Sethi *et al.*[19] (14.7 years vs. 6.9 years). Our data also show that a knowledge gap remains among pediatric MN antigens given that 36% of the larger cohort was negative for the known 6 antigens stained.

Our data have some important clinical implications despite some limitations of the study. EXT1+ membranous nephropathy has been associated with autoimmune diseases, most commonly systemic lupus erythematosus.[13] Our smaller cohort's 5 patients who were EXT1 positive did not have evidence of autoimmune disease or systemic lupus erythematosus at the time of diagnosis and until the most recent follow-up. One patient was diagnosed with mixed connective tissue disease within 1 year after the diagnosis of PMN. However, EXT+ membranous nephropathy may be a harbinger of later development of autoimmune disease, and membranous nephropathy can be the first sign of lupus nephritis in a subset of patients.[30] In our cohort, EXT1+ patients had a favorable clinical outcome with 100% of children entering complete remission. This is similar to what has been reported in adult patients, where EXT1/2+ patients have a better kidney prognosis than EXT1/2-patients with lupus nephritis.[38–40]

In addition, in our cohort, treatment with immunosuppression and antigen-negative biopsies tended to be poor prognostic factors. Patients who were treated with immunosuppression tended to be less likely to enter a complete or partial remission than the patients who were treated with conservative management, although the difference was not statistically significant. However, when interpreting these results, we must be cognizant of confounding by indication such that these patients may appear to have had a worse outcome simply because their disease was more severe at presentation. Unfortunately, our study does not have data regarding the time to initiation of therapy relative to the time of presentation or diagnosis. Therefore, for those children with severe disease who are at risk of complications from nephrotic syndrome, it is reasonable to consider a short course of immunosuppression, consistent with the Kidney Disease Improving Global Outcomes guidelines.[31,41] Long courses of immunosuppression, particularly prednisone, may expose the patient to untoward side effects without evidence of significant benefit.

Limitations of this study include its retrospective study design and small sample size of the clinical cohort. There is a lack of serologic anit-PLA2R data because the commercial assay has only become more

readily available in recent years. In addition, there was no uniform treatment protocol for the patients. All patients were treated at the discretion of the primary provider, which makes therapeutic conclusions for this study difficult to ascertain.

Conclusion

Approximately, 60% to 65% of children with PMN have antigen positivity by immunostaining. In our study, PLA2R and EXT1 were the most common novel antigens that were identified. EXT1 is more common in our pediatric cohort than in adult cohorts and similarly often portends a favorable clinical outcome. However, regardless of antigen subtype, the short-term kidney prognosis in PMN was favorable. More studies are needed to further our understanding of the histopathologic characteristics and clinical correlation of disease in pediatric patients.

DISCLOSURE

All the authors declared no competing interests.

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SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

STROBE Statement.

REFERENCES

1. Couser WG. Primary membranous nephropathy. *Clin J Am Soc Nephrol*. 2017;12:983–997. https://doi.org/10.2215/CJN.11761116
2. Valentini RP, Mattoo TK, Kapur G, Imam A. Membranous glomerulonephritis: treatment response and outcome in children. *Pediatr Nephrol*. 2009;24:301–308. https://doi.org/10.1007/s00467-008-1005-9
3. Moxey-Mims MM, Stapleton FB, Feld LG. Applying decision analysis to management of adolescent idiopathic nephrotic syndrome. *Pediatr Nephrol*. 1994;8:660–664. https://doi.org/10.1007/BF00869080
4. Chen A, Frank R, Vento S, et al. Idiopathic membranous nephropathy in pediatric patients: presentation, response to therapy, and long-term outcome. *BMC Nephrol*. 2007;8:11. https://doi.org/10.1186/1471-2369-8-11
5. Kleinknecht C, Levy M, Gagnadoux MF, Habib R. Membranous glomerulonephritis with extra-renal disorders in children. *Med (Baltim)*. 1979;58:219–228. https://doi.org/10.1097/00005792-197905000-00002
6. Churg J, Habib R, White RH. Pathology of the nephrotic syndrome in children: a report for the International Study of Kidney Disease in Children. *Lancet*. 1970;760:1299–1302. https://doi.org/10.1016/s0140-6736(70)91905-7
7. Ayalon R, Beck LH Jr. Membranous nephropathy: not just a disease for adults. *Pediatr Nephrol*. 2015;30:31–39. https://doi.org/10.1007/s00467-013-2717-z
8. Menon S, Valentini RP. Membranous nephropathy in children: clinical presentation and therapeutic approach. *Pediatr Nephrol*. 2010;25:1419–1428. https://doi.org/10.1007/s00467-009-1324-5
9. Mubarak M, Kazi JI, Lanewala A, Hashmi S, Akhter F. Pathology of idiopathic nephrotic syndrome in children: are the adolescents different from young children? *Nephrol Dial Transplant*. 2012;27:722–726. https://doi.org/10.1093/ndt/gfr221
10. Hogg RJ, Silva FG, Berry PL, Wenz JE. Glomerular lesions in adolescents with gross hematuria or the nephrotic syndrome. Report of the Southwest pediatric nephrology study group. *Pediatr Nephrol*. 1993;7:27–31. https://doi.org/10.1007/BF00861557
11. Beck LH Jr, Bonegio RG, Lambeau G, et al. M-type phospholipase A2 receptor as target antigen in idiopathic membranous nephropathy. *N Engl J Med*. 2009;361:11–21. https://doi.org/10.1056/NEJMoa0810457
12. Tomas NM, Beck LH Jr, Meyer-Schwesinger C, et al. Thrombospondin type-1 domain-containing 7A in idiopathic membranous nephropathy. *N Engl J Med*. 2014;371:2277–2287. https://doi.org/10.1056/NEJMoa1409354
13. Sethi S, Madden BJ, Debiec H, et al. Exostosin 1/exostosin 2-associated membranous nephropathy. *J Am Soc Nephrol*. 2019;30:1123–1136. https://doi.org/10.1681/ASN.2018080852
14. Caza TN, Hassen SI, Dvanajscak Z, et al. NELL1 is a target antigen in malignancy-associated membranous nephropathy. *Kidney Int*. 2021;99:967–976. https://doi.org/10.1016/j.kint.2020.07.039
15. Sethi S, Madden B, Debiec H, et al. Protocadherin 7-associated membranous nephropathy. *J Am Soc Nephrol*. 2021;32:1249–1261. https://doi.org/10.1681/ASN.2020081165
16. Bobart SA, Tehranian S, Sethi S, et al. A target antigen-based approach to the classification of membranous nephropathy. *Mayo Clin Proc*. 2021;96:577–591. https://doi.org/10.1016/j.mayocp.2020.11.028
17. Caza TN, Hassen SI, Kuperman M, et al. Neural cell adhesion molecule 1 is a novel autoantigen in membranous lupus nephritis. *Kidney Int*. 2021;100:171–181. https://doi.org/10.1016/j.kint.2020.09.016
18. Ronco P, Debiec H. Membranous nephropathy: current understanding of various causes in light of new target antigens. *Curr Opin Nephrol Hypertens*. 2021;30:287–293. https://doi.org/10.1097/MNH.0000000000000697
19. Sethi S, Debiec H, Madden B, et al. Semaphorin 3B-associated membranous nephropathy is a distinct type of disease predominantly present in pediatric patients. *Kidney Int*. 2020;98:1253–1264. https://doi.org/10.1016/j.kint.2020.05.030
20. Caza TN, Hassen SI, Kenan DJ, et al. Transforming growth factor beta Receptor 3 (TGFBR3)-associated membranous nephropathy. *Kidney360*. 2021;2:1275–1286. https://doi.org/10.34067/KID.0001492021
21. Al-Rabadi LF, Caza T, Trivin-Avillach C, et al. Serine protease HTRA1 as a novel target antigen in primary membranous

nephropathy. *J Am Soc Nephrol*. 2021;32:1666–1681. https://doi.org/10.1681/ASN.2020101395

22. Sethi SM, Benjamin Moura, et al. Membranous nephropathy in syphilis is associated with neuron-derived neurotrophic factor. *J Am Soc Nephrol*. 2023;34:374–384. https://doi.org/10.1681/ASN.0000000000000061
23. Sethi S, Madden B, Casal Moura M, et al. Hematopoietic stem cell transplant-membranous nephropathy is associated with protocadherin FAT1. *J Am Soc Nephrol*. 2022;33:1033–1044. https://doi.org/10.1681/ASN.2021111488
24. Caza TN, Storey A, Hassen SI, et al. Discovery of seven novel putative antigens in membranous nephropathy and membranous lupus nephritis identified by mass spectrometry. *Kidney Int*. 2023;103:593–606. https://doi.org/10.1016/j.kint.2023.01.001
25. Tsukahara H, Takahashi Y, Yoshimoto M, et al. Clinical course and outcome of idiopathic membranous nephropathy in Japanese children. *Pediatr Nephrol (Berlin, Germany)*. 1993;7:387–391. https://doi.org/10.1007/BF00857546
26. Kumar V, Varma AK, Nada R, et al. Primary membranous nephropathy in adolescence: a prospective study. *Nephrol (Carlton)*. 2017;22:678–683. https://doi.org/10.1111/nep.12835
27. Kumar V, Ramachandran R, Kumar A, et al. Antibodies to m-type phospholipase A2 receptor in children with idiopathic membranous nephropathy. *Nephrology (Carlton)*. 2015;20:572–575. https://doi.org/10.1111/nep.12478
28. Malatesta-Muncher R, Eldin KW, Beck LH Jr, Michael M. Idiopathic membranous nephropathy in children treated with rituximab: report of two cases. *Pediatr Nephrol*. 2018;33:1089–1092. https://doi.org/10.1007/s00467-018-3923-5
29. Latham P, Poucell S, Koresaar A, Arbus G, Baumal R. Idiopathic membranous glomerulopathy in Canadian children: a clinicopathologic study. *J Pediatr*. 1982;101:682–685. https://doi.org/10.1016/s0022-3476(82)80290-4
30. Miller P, Lei L, Charu V, Higgins J, Troxell M, Kambham N. Clinicopathologic features of non-lupus membranous nephropathy in a pediatric population. *Pediatr Nephrol*. 2022;37:3127–3137. https://doi.org/10.1007/s00467-022-05503-7
31. O'Shaughnessy MM, Troost JP, Bomback AS, et al. Treatment patterns among adults and children with membranous nephropathy in the cure glomerulonephropathy network (CureGN). *Kidney Int Rep*. 2019;4:1725–1734. https://doi.org/10.1016/j.ekir.2019.09.005
32. Andersson C, Johnson AD, Benjamin EJ, Levy D, Vasan RS. 70-year legacy of the Framingham Heart Study. *Nat Rev Cardiol*. 2019;16:687–698. https://doi.org/10.1038/s41569-019-0202-5
33. Schwartz GJ, Muñoz A, Schneider MF, et al. New equations to estimate GFR in children with CKD. *J Am Soc Nephrol*. 2009;20:629–637. https://doi.org/10.1681/ASN.2008030287
34. Thompson A, Cattran DC, Blank M, Nachman PH. Complete and partial remission as surrogate end points in membranous nephropathy. *J Am Soc Nephrol*. 2015;26:2930–2937. https://doi.org/10.1681/ASN.2015010091
35. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research Electronic Data Capture (REDCap)–a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform*. 2009;42:377–381. https://doi.org/10.1016/j.jbi.2008.08.010
36. Harris PA, Taylor R, Minor BL, et al. The REDCap consortium: building an international community of software platform partners. *J Biomed Inform*. 2019;95:103208. https://doi.org/10.1016/j.jbi.2019.103208
37. Cossey LN, Walker PD, Larsen CP. Phospholipase A2 receptor staining in pediatric idiopathic membranous glomerulopathy. *Pediatr Nephrol*. 2013;28:2307–2311. https://doi.org/10.1007/s00467-013-2574-9
38. Li H, Lan P, Yu X, et al. Analysis of the expression of exostosins and clinicopathological features in membranous lupus nephritis in a Chinese cohort. *Kidney Int Rep*. 2022;7:2295–2298. https://doi.org/10.1016/j.ekir.2022.07.164
39. Ravindran A, Casal Moura M, Fervenza FC, et al. In patients with membranous lupus nephritis, exostosin-positivity and exostosin-negativity represent two different phenotypes. *J Am Soc Nephrol*. 2021;32:695–706. https://doi.org/10.1681/ASN.2020081181
40. Saïdi M, Brochériou I, Estève E, et al. The exostosin immunohistochemical status differentiates lupus membranous nephropathy subsets with different outcomes. *Kidney Int Rep*. 2021;6:1977–1980. https://doi.org/10.1016/j.ekir.2021.04.025
41. Kidney Disease: Improving Global Outcomes (KDIGO) Glomerular Diseases Work Group. KDIGO 2021 clinical practice guideline for the management of glomerular diseases. *Kidney Int*. 2021;100:S1–s276.