Glomerular Diseases

Research Article

Glomerular Dis 2022;2:75–82
DOI: 10.1159/000520641

Received: August 13, 2021
Accepted: October 29, 2021
Published online: November 2, 2021

Anti-Phospholipase A2 Receptor in Nonlupus Patients with Membranous Nephropathy and Crescents

Yiqin Zuo[a, b] Livia Barreira Cavalcante[c] James Monroe Smelser[d]
Neil Sanghani[e] Jamie P. Dwyer[e] Julia Breyer Lewis[e] Agnes B. Fogo[a, f, g]

[a]Department of Pathology, Microbiology, and Immunology, Vanderbilt University Medical Center, Nashville, TN, USA; [b]Department of Pathology and Laboratory Medicine, University of Miami, Miami, FL, USA; [c]Pathology Department, University of São Paulo School of Medicine, São Paulo, Brazil; [d]Huntsville Renal Clinic PC, Huntsville, AL, USA; [e]Division of Nephrology and Hypertension, Vanderbilt University Medical Center, Nashville, TN, USA; [f]Department of Medicine, Vanderbilt University Medical Center, Nashville, TN, USA; [g]Division of Pediatric Nephrology, Vanderbilt University Medical Center, Nashville, TN, USA

Keywords
Membranous nephropathy · Crescents · Anti-phospholipase A2 receptor

Abstract

Introduction: Anti-phospholipase A2 receptor (PLA2R) is detected in approximately 70% of biopsies of "primary" membranous nephropathy (MN). Crescents in MN in nonlupus patients suggest additional injury, such as antineutrophil cytoplasmic antibody (ANCA) or anti-glomerular basement membrane (anti-GBM)-associated glomerulonephritis and are postulated to reflect injury by a mechanism that unmasks cryptic epitopes leading to the second autoantibody. ***Methods:*** We studied PLA2R staining in nonlupus patients with MN and crescents. Native renal biopsies in 16 nonlupus patients with MN and crescents were stained for PLA2R. ***Results:*** The patients included 5 women and 11 men, with mean age 61 years and elevated serum creatinine (mean 4.68 mg/dL). Hematuria and proteinuria (mean 4.97 g/day) were documented in 13 patients. Two patients had positive serum anti-GBM antibody. Nine of 11 patients tested for ANCA were positive, with p-ANCA ($n = 4$), c-ANCA ($n = 2$), or both ($n = 1$), with 2 not specified. On average, 27% of glomeruli had crescents. One patient had an initial biopsy with MN, 4 years later had MN with crescent, and 7 years later had rebiopsy with persistent MN with crescents. One patient had ANCA-associated vasculitis, and 5 years later had MN and crescent. The remaining 14 patients had concurrent diagnoses of MN and crescents. PLA2R was positive in 5 cases, 3 with ANCA positivity, 2 with unknown ANCA status, and none with anti-GBM disease. The patient with initial MN preceding crescent was PLA2R positive; the patient with initial ANCA-associated vasculitis preceding MN was PLA2R negative. ***Conclusions:*** Most patients (64%) presented with concomitant MN and crescents, with rare occurrence of an initial disease process followed later by the second injury. PLA2R was positive in 31% of patients, suggesting most are secondary MN. Further study to determine the cryptic epitopes may shed light on the triggering mechanisms for these rare but unlikely coincidental glomerular injuries.

karger@karger.com
www.karger.com/gdz

Correspondence to:
Agnes B. Fogo, agnes.fogo@vanderbilt.edu

Introduction

Membranous nephropathy (MN) is one of the most common causes of nephrotic range proteinuria in adults [1]. Approximately 80% of cases are so-called "primary," without evident triggering etiology and the remainder are so-called "secondary," associated with, e.g., systemic diseases, drug or exogenous agent exposures. Phospholipase A2 receptor (PLA2R) staining is positive in kidney biopsies in about 70% of so-called primary MN, with an increasing number of endogenous antigens recognized as the target in the PLA2R-negative cases [2–5]. Crescents in primary MN are unusual, and suggest lupus nephritis, or other additional injury, such as antineutrophil cytoplasmic antibody (ANCA)- or anti-glomerular basement membrane (anti-GBM)-associated glomerulonephritis [6, 7]. The coexistence of nonlupus MN with crescents has been postulated to reflect initial injury by one of these mechanisms that may then unmask cryptic epitopes and lead to the second autoantibody [8]. Although a few case reports and case series describe the clinical presentation, pathological findings, and clinical outcomes of this unusual coexistence of lesions [6, 8–12], the role of anti-PLA2R and the possible causal relationship between these two injury processes remain unclear. Here, we studied 16 nonlupus patients with MN and crescents, PLA2R staining, and ANCA and anti-GBM antibody status. Our case series represents the largest number of such MN patients including those with ANCA or anti-GBM antibody studied for PLA2R staining. Additionally, our cohort offered the opportunity to investigate rare patients where MN and the crescentic injury were documented at different time points. We hypothesized that if crescentic injury was the initial insult, the ensuing MN would likely be PLA2R negative, and conversely if MN was the first injury, it more commonly would be PLA2R positive, reflecting the more common form of MN.

Materials and Methods

With approval from the Institutional Review Board of Vanderbilt University, the archives of the renal pathology laboratory at the Vanderbilt University Medical Center were searched for native kidney biopsy cases from January 2008 to March 2018 to identify biopsies with MN with any crescentic lesions. Patients with systemic lupus erythematosus were excluded. Patients' medical records including evidence of systemic vasculitis, medication history, and laboratory results, including ANCA specificity, serum creatinine, urinalysis, proteinuria, treatment, and outcome were reviewed.

Standard techniques of renal biopsies processed at our institution included light microscopy (LM), immunofluorescence (IF), and electron microscopy (EM). For LM, 2-μm sections from formalin-fixed paraffin-embedded tissue were stained with hematoxylin and eosin, periodic acid-Schiff (PAS), and Jones' methenamine silver. For IF, 4 μm cryostat sections were stained with polyclonal fluorescein isothiocyanate-conjugated antibodies to IgG, IgA, IgM, C3, C1q, kappa and lambda light chains and polyvalent antisera. IF intensity and pattern were described and semi-quantitatively scored by the pathologist at time of diagnosis on a scale of 0–3+. EM was performed in all cases with available tissue and examined by an experienced renal pathologist using a Philips FEI Morgagni transmission electron microscope.

Anti- PLA2R staining was performed for this study by IF on frozen sections, using a rabbit anti-PLA2R1 primary antibody (Sigma-Aldrich) and polyclonal goat anti-rabbit IgG (Life Technologies) as the secondary antibody. Biopsies of known PLA2R-positive MN were used as positive controls, while biopsies of lupus membranous nephritis were used as negative controls, with additional negative controls without primary antibody. PLA2R antibody staining was considered positive if it showed granular capillary loop staining in the same pattern as IgG. The extent of crescents and other lesions were evaluated independently of knowledge of the PLA2R staining.

Statistical analysis was performed using SPSS for Windows. Continuous variables are reported as the mean ± standard deviation. For all tests, statistical significance was assumed at $p < 0.05$.

Results

Clinical Presentation and Laboratory Findings

Our cohort study consisted of 16 patients including 5 women and 11 men. The clinical presentation, laboratory findings, treatment, and outcomes of these patients with MN and crescents are summarized in Table 1.

Three patients were black and 12 were white with 1 patient of unknown ethnicity. The mean age at biopsy was 61 years (range 22–81 years). Proteinuria and hematuria were present in all 13 patients in whom this assessment was documented. Nine of 13 patients had 24-h urine collections with mean proteinuria 4.97 g/day (range 0.31–8.40 g/day). The remaining 4 patients had 100 mg/dL, 300 mg/dL, >300 mg/dL, or "nephrotic range" proteinuria on urinalysis. All patients had elevated serum creatinine levels, mean 4.68 mg/dL (range 1.84–20.00 mg/dL). In 7 patients in whom baseline creatinine levels were available, the range of increase of serum creatinine over follow-up was from 1.25 to 5.75 fold.

Two patients had positive serum anti-GBM antibody tests. Nine of 11 patients tested had positive ANCA; among these, 4 had p-ANCA, 2 had c-ANCA, 1 had both positive, and 2 had ANCA type not specified. One patient (case 4) was serologically positive for hepatitis C. Antinuclear antibody was negative in the 5 patients tested, and anti-doublestranded DNA was negative in both patients tested.

Table 1. Clinical data

Patient	Age, years	Ethnicity	Gender	ANCA	Anti-GBM serology	Urine protein, mg/24 h or UA	Serum Cr, mg/dL
1	59	White	Male	Negative	Positive	5,500	2.8
2	61	White	Female	c-ANCA and MPO positive	Negative	8,000	8.0
3	46	Black	Male	Unknown	Unknown	8,400	2.7
4	56	Black	Male	Unknown	Unknown	7,000	2.4
5	63	Unknown	Female	c-ANCA positive	Unknown	300 mg/dL	4.1
6	47	White	Male	p-ANCA positive (1:160), MPO positive (58.5)	Negative	309.3	2.1
7	22	White	Male	Unknown	Unknown	>300 mg/dL	2.0
8	74	White	Male	Unknown	Markedly elevated	Unknown	20.0
9	73	White	Female	Unknown	Unknown	5,000	2.0
10	81	White	Male	P-ANCA positive	Negative	4,980	3.7
11	54	White	Male	Positive (5 yr ago), persistently elevated	Unknown	1,000	1.8
12	50	Black	Female	Negative	Unknown	Nephrotic	4.6
13	58	White	Male	p-ANCA (+)	Unknown	100 mg/dL	3.5
14	79	White	Female	PR3 (+)	Unknown	4,500	3.7
15	88	White	Male	p-ANCA (+)	Negative	Unknown	4.8
16	60	White	Male	ANCA (+)	Unknown	Unknown	6.6

ANCA, antineutrophil cytoplasmic antibodies; c-ANCA, cytoplasmic antineutrophil cytoplasmic antibodies; p-ANCA, perinuclear antineutrophil cytoplasmic antibodies; MPO, myeloperoxidase; PR3, proteinase 3; UA, urinalysis; Serum Cr, serum creatinine; GBMs, glomerular basement membranes.

Pathologic Findings

The renal biopsy features are summarized in Table 2. Diagnostic features of MN and crescentic lesions were present in all biopsies by study design. Fifteen glomeruli on average were present in the light microscopic sample (range 5–26). Crescents were present in 27% of glomeruli on average (range 5–89%), mostly cellular (54% of crescents, range 0–100%). Fibrinoid necrosis, evidenced by GBM breaks, karyorrhexis and fibrin, was present in 13% of glomeruli (range 0–56%). MN features were evident by LM in 11 cases, including pinpoint hole appearance and spike formation along the GBM on silver stains. None of the cases showed endocapillary hypercellularity. The degree of interstitial fibrosis with proportional tubular atrophy ranged from mild (affecting 1–25% of the cortex; 7 patients) to moderate (affecting 26–50% of the cortex, 7 patients) to severe (>50% of the cortex; 2 patients), on average 32% (range 10–65%). There was no necrotizing vasculitis in the arterioles or arteries.

IF demonstrated granular, segmental to global glomerular capillary loop positivity in all patients for IgG, and in all except 1 for kappa and lambda light chains, with the mean intensity for these staining for all patients 1.77, 1.19, and 1.06 (0–3+ scale), respectively. Of our 16 patients, 2 patients showed weaker than IgG level staining for IgA, 11 had weaker IgM, 14 had weaker C3, and 5 patients had weaker C1q, respectively. In the 2 patients with positive anti-GBM antibody, anti-GBM staining was evidenced by trace to 1+ linear capillary loop IgG staining in addition to 3+ granular capillary wall staining for IgG. None of the patients showed a full-house staining pattern, which would be suggestive of lupus nephritis. No extraglomerular staining for IgG was present.

EM was performed in all cases and showed characteristic features of MN with subepithelial and/or intramembranous electron dense deposits. Among these, 10 cases showed a global or near global pattern of deposits (involving >50% of the glomerular capillary loops). Foot process effacement was on average 73% (range 10–100%). Mesangial deposits were identified in 10 cases, ranging from very rare small to scattered medium-sized, which are commonly reported in the setting of pauci-immune necrotizing crescentic glomerulonephritis. Four cases showed rare small to occasional medium-sized subendothelial deposits. None of the cases demonstrated tubuloreticular inclusions or tubular basement membrane deposits.

ANCA versus Anti-GBM Associated Cases and Time Course

We then compared the 2 patients with positive anti-GBM antibody and the group with positive ANCA (9 patients). As expected, the patients with positive anti-GBM antibody had more extensive crescents including both

Glomerular Dis 2022;2:75–82
DOI: 10.1159/000520641

Table 2. Renal pathology findings

Patient	LM						IF (intensity) and PLA2R								EM		
	glom, *n*	GS, %	% glom with crescents	CC/crescent, %	spikes/ holes	int. fibrosis, %	IgG	IgA	IgM	C3	C1q	κ	λ	PLA2R	FPE, %	mesangial deposits	subendo deposits
1*	26	7.7	50	69	Spikes and holes	50	3.0	0.0	0.5	2.0	0.3	2.0	2.0	–	100	Scattered small to medium	Absent
2	9	44.4	22	0	Spikes and holes	45	0.8	0.0	0.5	0.5	0.5	0.8	0.8	–	60	Rare small	Very rare
3	12	0.0	17	100	Spikes and holes	15	3.0	1.0	0.0	2.0	0.0	1.0	1.0	+	100	Very rare small	Absent
4	9	22.2	11	0	Holes	10	1.0	0.0	1.0	1.0	0.0	1.0	1.0	–	100	Rare	Scattered small
5	25	28.0	20	100	Absent	40	0.5	0.0	0.0	0.0	0.0	0.0	0.0	–	10	Absent	Absent
6	23	47.8	17	75	Holes	30	2.0	0.0	1.0	1.5	0.0	0.8	0.8	–	70–80	Rare small	Absent
7#	16	6.3	38	0	Absent	20	3.0	0.0	0.5	0.5	0.0	2.0	1.5	–	70–80	Absent	Absent
8*	9	11.1	89	100	Absent	65	2.0	0.0	0.0	0.8	0.3	2.0	2.0	–	30–40	Absent	Absent
9	12	25.0	8	100	Holes	10	0.3	0.3	0.3	0.3	0.0	0.3	0.8	+	100	Very rare	Absent
10	16	37.5	56	89	Holes	15	2.5	0.0	0.0	2.5	0.3	2.5	2.5	–	90	Occasional medium	Occasional medium
11	12	16.7	8	0	Holes	20	3.0	0.0	0.8	1.0	0.0	3.0	1.0	–	80–90	Occasional medium	Absent
12	21	23.8	5	0	Spikes and holes	30	2.0	0.0	0.5	0.5	0.0	0.5	0.5	–	>90	Absent	Absent
13	24	4.2	25	50	Absent	25	0.5	0.0	0.5	0.5	0.5	0.5	0.5	+	30–40	Absent	Absent
14	13	15.4	31	25	Absent	40	3.0	0.0	1.0	1.0	0.0	1.5	1.5	+	80	Scattered medium	Very rare sliver-like
15	5	0.0	20	100	Holes	60	1.0	0.0	0.0	0.0	0.0	0.8	0.8	–	70	Absent	Absent
16	14	35.7	7	100	Spikes and holes	40	0.8	0.0	0.5	0.5	0.0	0.5	0.5	+	40–50	Rare small	Absent

GS, global glomerulosclerosis; CC, cellular crescent; Int. fibrosis, interstitial fibrosis; κ, kappa light chain; λ, lambda light chain; FPE, foot process effacement; LM, light microscopy; EM, electron microscopy; IF, immunofluorescence; PLA2R, phospholipase A2 receptor; GBMs, glomerular basement membranes. * Trace to 1+ linear capillary loop IgG staining in addition to 3+ granular capillary wall staining for IgG. # Unusual linear GBM staining.

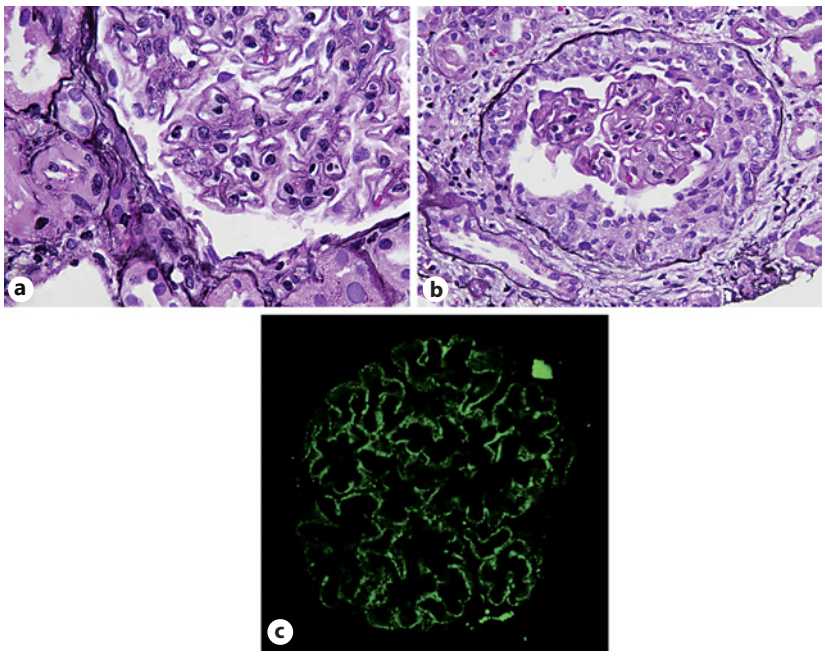

Fig. 1. Kidney biopsy of Case 9. **a** A representative glomerulus shows the rigid GBM with rare pinpoint hole appearance, characteristic of early MN (Jones' silver stain, ×400). **b** A cellular crescent is present (Jones' silver stain, ×200). **c** A representative glomerulus shows finely granular staining along the glomerular capillary loops for PLA2R (anti-PLA2R IF stain, ×400). MN, membranous nephropathy; IF, immunofluorescence; PLA2R, phospholipase A2 receptor; GBMs, glomerular basement membranes.

cellular and fibrocellular crescents, involving 70 ± 28% of glomeruli, compared to the patients with positive ANCA, 23 ± 15% ($p = 0.006$). The percentage of cellular crescents or the percentage of crescents with fibrinoid necrosis did not differ between groups ($p = 0.46$ and 0.65, respectively).

Next, we studied the sequence of MN and development of crescents. The most common pattern was detection of concurrent MN with crescents at the time of biopsy, observed in 14 patients. Among these, 2 had positive anti-GBM antibody and 8 had positive ANCA. Of note, 1 patient (case 9) had an initial biopsy of MN evidenced by spikes along the GBM with classic polyclonal IgG granular capillary loop staining without any crescents. A second biopsy 5 years later showed a cellular crescent in addition to persistent MN with unknown status of ANCA and anti-GBM antibody at that time (Fig. 1). A third biopsy 7 years after initial presentation showed MN features and 2 fibrocellular crescents. Another patient (case 11) had a clinical diagnosis of vasculitis with positive ANCA 5 years prior to a kidney biopsy, which demonstrated MN with 1 fibrous crescent.

PLA2R Staining

Five of the 16 cases showed PLA2R positivity in the kidney biopsies. Neither of the 2 cases with positive anti-GBM antibody had positive PLA2R. Among the 14 cases with concurrent detection of MN and crescents, 4 cases were PLA2R positive, 3 of these with positive ANCA, and 10 were PLA2R negative, and 5 with positive ANCA. In addition, 2 cases with unknown ANCA status were PLA2R positive. The biopsy in the patient (case 9) with initial diagnosis of MN, and MN with crescent 5 years later showed positive PLA2R staining. The biopsy in the patient (case 11) with positive ANCA and vasculitis for 5 years prior to the renal biopsy with MN and crescent was PLA2R negative (Fig. 2).

Clinical Follow-Up

Follow-up on most of these biopsies was not available, as most were outside referral biopsies sent to us for primary diagnosis. In patient 9, repeat biopsy 2 years after a second biopsy showed persistent MN and crescents, with increased chronicity with fibrocellular crescents and increased interstitial fibrosis and tubular atrophy after initial treatment with unspecified immunosuppression.

Patient 1 presented with a 1-month history of hematuria, proteinuria (5.5 g/day), fatigue, and fever and had increased serum creatinine (2.8 mg/dL, from baseline creatinine 0.9 mg/dL 4 months earlier). After biopsy, this patient was treated with plasmapheresis, then cytoxan for slightly more than a year, and then switched to Imuran

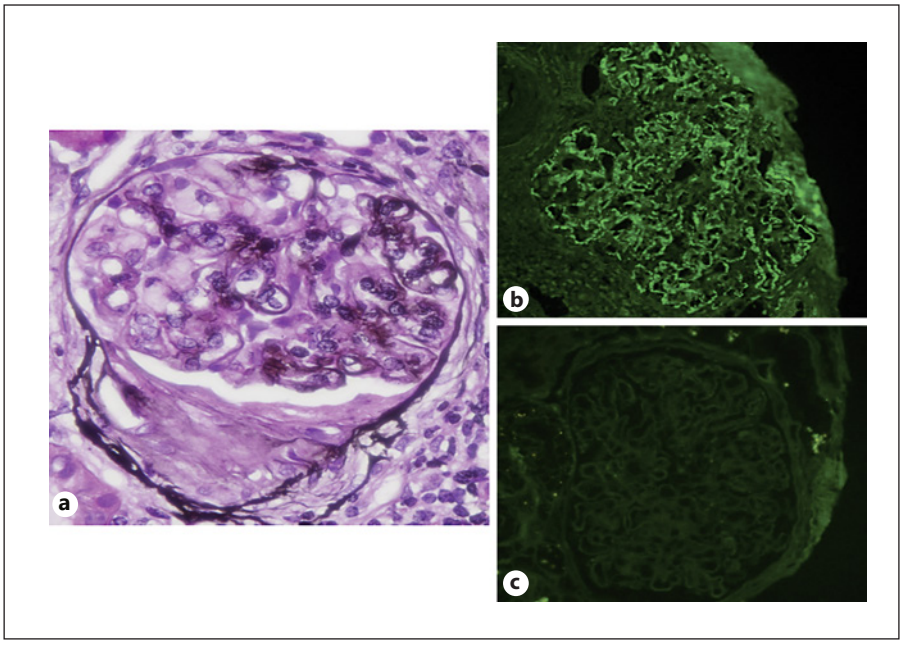

Fig. 2. Kidney biopsy of Case 11: **a** A fibrous crescent is present (Jones' silver stain, ×400). **b** A representative glomerulus shows finely granular staining along the glomerular capillary loop for IgG (IF stain, ×400). **c** A representative glomerulus shows negative staining for PLA2R (anti-PLA2R IF stain, ×400). IF, immunofluorescence; PLA2R, phospholipase A2 receptor.

for 1 year, and has since been off treatment. The patient has remained in remission with mild proteinuria (0.28 g/day) and serum creatinine 1.4 mg/dL at last known follow-up 8 years after biopsy.

Patient 13 received monthly cyclophosphamide 1 g i.v. for 6 months, then azathioprine for maintenance therapy for 2.5 years. His serum creatinine has remained near 2 mg/dL without hematuria or proteinuria.

Patient 15 presented with edema, weight loss, and increased serum creatinine. After biopsy results, he was treated with steroids and oral cyclophosphamide. The patient then showed monoclonal gammopathy of undetermined significance, with no monoclonal component in the biopsy. Initially, serum creatinine improved from 5 mg/dL to 2.7 mg/dL, but 1 month after biopsy had marked edema and dyspnea with little response to aggressive therapy with steady worsening of renal function. He and his family eventually opted for comfort care only and declined dialysis.

Discussion

In the absence of evidence of systemic lupus erythematosus, MN with crescentic lesions is rarely encountered. When present, these findings suggest the possibility of a superimposed disease process, either additional anti-GBM disease or ANCA-associated necrotizing crescentic glomerulonephritis. Little is known about the potential causal relationship between the injury processes leading to MN and crescents. In this study, we investigated a cohort of nonlupus MN with crescents and assessed PLA2R staining and ANCA and anti-GBM antibody status. Our study is the first and largest series to date with PLA2R staining of such MN patients with crescents, including those with ANCA or anti-GBM antibody. Our data demonstrate that 64% of the patients with concurrent MN and crescents had coexisting ANCA-associated disease, while 14% of the patients with concurrent MN and crescents had coexisting anti-GBM disease. PLA2R was negative in 69% of patients, suggesting most cases are likely so-called secondary MN. Importantly and uniquely, in 2 of our patients, we shed additional light on the potential interaction of these injury mechanisms, where either MN or ANCA-related disease was proven to be the initial injury.

In 1974, MN combined with anti-GBM antibody glomerulonephritis was first recognized by Klassen et al. [13] in a patient with biopsy-proven MN followed by an acute decline in renal function and death. The autopsy demonstrated crescentic lesions with IgG linear staining along the GBM in addition to classic MN. The authors postulated that the MN induced release of antigenic GBM fragments, which subsequently provoked an immune response with formation of antibodies against the GBM. Since then, 60–70 cases have been described so far [14–17]. In some cases, MN was followed by anti-GBM glo-

merulonephritis; in some, anti-GBM glomerulonephritis was followed by MN; and the remaining showed simultaneous detection of findings of MN and anti-GBM disease.

In our study, 2 patients demonstrated simultaneous findings of MN and anti-GBM disease, with fair prognosis, similar to previous studies [17]. In the most recent case series of 12 patients with anti-GBM disease and MN, concurrent detection of both patterns of injury was the most common presentation and only 1 of 5 of the patients tested for PLA2R was positive [14]. Neither of the 2 cases in our study with MN and anti-GBM disease showed positive PLA2R. In addition, the scattered small mesangial deposits by EM in one of our cases, although not specific, could suggest a secondary MN. Other possibilities of negative PLA2R in a MN case without specific exogenous trigger include autoantibodies to additional uncommon antigens of MN such as thrombospondin type-1 domain containing 7A, exostosin-1/2, neural epidermal growth factor-like 1 protein, or other novel antigens, or low concentration of PLA2R below the detection level. Recently, a mouse model study demonstrated that the linear peptide of human alpha3(IV)NC1 could induce clinical and histopathological features of MN in DBA/1 mice, which might provide clues to the mechanism underlying development of MN in combination with anti-GBM disease [18].

MN with superimposed ANCA disease is also a rare phenomenon. So far approximately 50 cases have been reported, including 2 larger cohort studies [10, 19–21]. In one of these larger series, 14 cases were identified, all with heavy proteinuria, active urine sediment, and acute kidney injury with 50% of patients reaching end-stage kidney disease or death. One patient had biopsy-proven MN 7 months prior to MN with crescent formation. Another patient had granulomatosis with polyangiitis for 1 year before a biopsy showed MN with crescents. The remaining cases showed simultaneous detection of MN and crescentic lesions. No PLA2R or IgG subclasses studies were performed [10]. In another large series, 13 cases were identified, all with simultaneous detection of the combined lesions. IgG subclasses and PLA2R studies were conducted in 7 of these cases, and only 2 were positive for PLA2R [19]. A most recent UK study of 7 cases with MN and crescents with positive ANCA showed negative PLA2R staining [11]. Our cohort demonstrated similar findings, in that most patients (6 of 9) with ANCA were negative for PLA2R, indicating more likely a secondary MN process. Combined with the published series above, the results were similar (18 of 22 patients with MN and crescents and ANCA showing negative PLA2R).

Interestingly, in our study, there was rare documented occurrence of an initial disease process followed later by the second injury. These rare cases include MN developing first in one patient, and ANCA disease first in another patient. The PLA2R findings in these 2 patients are of further interest. Thus, our patient #11 had a positive ANCA and a clinical diagnosis of vasculitis 5 years prior to a renal biopsy showing PLA2R-negative MN with a fibrous crescent. We postulate that this initial crescentic injury damaged the capillary wall, and thus may have played a role in initiating a second autoantibody causing MN, which was PLA2R negative, not as seen in most cases of usual "primary" MN. In contrast, patient #9 had biopsy-proven PLA2R positive MN preceding a second biopsy 5 years later with crescentic injury. We postulate that in this patient, the MN injury which preceded the subsequent development of an additional crescentic process damaged the capillary wall, and thus may have played a role in initiating a second autoantibody causing a vasculitic injury. Although serological studies for ANCA and anti-GBM antibodies were not done, the very limited crescents and lack of detection of anti-GBM staining in the biopsy support that the added crescentic process likely was an ANCA-associated type vasculitic injury.

The remaining 14 cases of our study revealed concurrent MN and crescents at the time of biopsy, including biopsies with coexisting anti-GBM disease or coexisting ANCA-associated disease. One of these biopsies, patient #4 with positive hepatitis C and unknown ANCA and anti-GBM antibody serologies showed scattered subendothelial deposits by EM, supporting a secondary etiology of the predominantly MN pattern of injury. This MN pattern injury was also PLA2R negative. Our case series is limited by relatively small size of these rare double injury pattern biopsies, lack of follow-up information of all patients, and lack of testing for IgG subclasses and other potential antigens of MN in the PLA2R-negative cases.

In summary, in our study, we present the largest number of such patients studied for PLA2R staining, rare patients where MN and the crescentic injury were documented at different time points. We speculate that individuals predisposed to develop one type of autoimmune disease may have a second such disease. Such multiple processes may relate to specific HLA alleles or other immune regulatory predispositions to autoimmune disease(s) which could potentially trigger unmasking of cryptic epitopes. Further study to determine the cryptic epitopes may shed light on the triggering mechanisms for these rare but unlikely coincidental glomerular injuries.

Acknowledgments

We thank Drs. David Bains, Chanty Davis, Chirag Faldu, Susan Francisco, Lorraine Cho Chung Hing, Muhammad R. Ishaque, Amin Kamyar, Juan Nunez, Kiran Padigala, Bharat Patel, Nilesh Patel, and Amish Shah, for giving us the privilege of diagnosing the kidney biopsies and thus being involved in the care of their patients.

Statement of Ethics

This study is in compliance with the guidelines for human studies and was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. The study was approved by the Institutional Review Board of Vanderbilt University (IRB #040174 Renal Pathology) and no personal identifying information was included. The written informed consent from the study was not required since this study was considered minimal risk/accessing follow-up clinical data from procedures that subject would undergo as part of clinical care and is not subject to FDA regulations 21 CFR 56.

Conflict of Interest Statement

The authors have no conflicts of interest to disclose.

Funding Sources

This research did not receive any specific Grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author Contributions

Yiqin Zuo contributed to study concept, acquisition of data, analysis and interpretation of data, drafting of the manuscript, and statistical analysis. Livia Barreira Cavalcante contributed to acquisition and analysis of data. James Monroe Smelser contributed to acquisition of data and follow-up study. Neil Sanghani contributed to acquisition of data and follow-up study. Jamie P. Dwyer contributed to acquisition of data and follow-up study. Julia Breyer Lewis contributed to acquisition of data and follow-up study, and revising of the manuscript. Agnes Fogo contributed to study concept and design, analysis and interpretation of data, drafting and revising of the manuscript. All authors reviewed and approved the final version of the manuscript as submitted and agreed to be accountable for all aspects of the work.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

References

1 Couser WG. Primary membranous nephropathy. Clin J Am Soc Nephrol. 2017;12(6):983–97.
2 Beck LH Jr, Bonegio RG, Lambeau G, Beck DM, Powell DW, Cummins TD, et al. M-type phospholipase A2 receptor as target antigen in idiopathic membranous nephropathy. N Engl J Med. 2009;361:11–21.
3 Hanset N, Aydin S, Demoulin N, Cosyns JP, Castanares-Zapatero D, Crott R, et al. Podocyte antigen staining to identify distinct phenotypes and outcomes in membranous nephropathy: a retrospective multicenter cohort study. Am J Kidney Dis. 2020;76:624–35.
4 Sethi S. New "antigens" in membranous nephropathy. J Am Soc Nephrol. 2021;32(2):268–78.
5 Sethi S, Debiec H, Madden B, Vivarelli M, Charlesworth MC, Ravindran A, et al. Semaphorin 3B-associated membranous nephropathy is a distinct type of disease predominantly present in pediatric patients. Kidney Int. 2020;98:1253–64.
6 Balafa O, Kalaitzidis R, Liapis G, Xiromeriti S, Zarzoulas F, Baltatzis G, et al. Crescentic glomerulonephritis and membranous nephropathy: a rare coexistence. Int Urol Nephrol. 2015;47:1373–7.
7 Saito M, Komatsuda A, Sato R, Saito A, Kaga H, Abe F, et al. Clinicopathological and long-term prognostic features of membranous nephropathy with crescents: a Japanese single-center experience. Clin Exp Nephrol. 2018;22:365–76.
8 Basford AW, Lewis J, Dwyer JP, Fogo AB. Membranous nephropathy with crescents. J Am Soc Nephrol. 2011;22:1804–8.
9 Alawieh R, Brodsky SV, Satoskar AA, Nadasdy T, Parikh SV, Rovin B, et al. Membranous nephropathy with crescents. Kidney Int Rep. 2020;5:537–41.
10 Nasr SH, Said SM, Valeri AM, Stokes MB, Masani NN, D'Agati VD, et al. Membranous glomerulonephritis with ANCA-associated necrotizing and crescentic glomerulonephritis. Clin J Am Soc Nephrol. 2009;4:299–308.
11 Nikolopoulou A, Huang-Doran I, McAdoo SP, Griffith ME, Cook HT, Pusey CD. Membranous glomerulonephritis with crescents. Kidney Int Rep. 2019;4:1577–84.
12 Rodriguez EF, Nasr SH, Larsen CP, Sethi S, Fidler ME, Cornell LD. Membranous nephropathy with crescents: a series of 19 cases. Am J Kidney Dis. 2014;64:66–73.
13 Klassen J, Elwood C, Grossberg AL, Milgrom F, Montes M, Sepulveda M, et al. Evolution of membranous nephropathy into anti-glomerular-basement-membrane glomerulonephritis. N Engl J Med. 1974;290:1340–4.
14 Ahmad SB, Santoriello D, Canetta P, Bomback AS, D'Agati VD, Markowitz G, et al. Concurrent anti-glomerular basement antibody disease and membranous nephropathy: a case series. Am J Kidney Dis. 2021;78(2):219–25.e1.
15 Cranfield A, Mathavakkannan S. Goodpasture's disease following extracorporeal shock wave lithotripsy: a case report & literature review. Clin Case Rep. 2015;3:160–4.
16 Nayak SG, Satish R. Crescentic transformation in primary membranous glomerulopathy: association with anti-GBM antibody. Saudi J Kidney Dis Transpl. 2007;18:599–602.
17 Ogawara A, Harada M, Ichikawa T, Fujii K, Ehara T, Kobayashi M. Coexistence of anti-glomerular basement membrane glomerulonephritis and membranous nephropathy in a female patient with preserved renal function. Tohoku J Exp Med. 2017;243:335–41.
18 Wang J, Wang M, Cui Z, Zhao MH. Epitope mapping of human alpha3(IV)NC1-induced membranous nephropathy in mice. Am J Nephrol. 2020;51:99–107.
19 Barrett CM, Troxell ML, Larsen CP, Houghton DC. Membranous glomerulonephritis with crescents. Int Urol Nephrol. 2014;46:963–71.
20 Fatima H, Siew ED, Dwyer JP, Paueksakon P. Membranous glomerulopathy with superimposed pauci-immune necrotizing crescentic glomerulonephritis. Clin Kidney J. 2012;5:587–90.
21 Gaber LW, Wall BM, Cooke CR. Coexistence of anti-neutrophil cytoplasmic antibody-associated glomerulonephritis and membranous glomerulopathy. Am J Clin Pathol. 1993;99:211–5.