

# Membranoproliferative Glomerulonephritis With Changing Immunofluorescence Pattern



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## INTRODUCTION

Membranoproliferative glomerulonephritis (MPGN) is a term most often used to describe a morphologic pattern of glomerular injury caused by etiologically distinct forms of glomerulonephritis (GN). MPGN can be primary/idiopathic disease (in which MPGN is used as a diagnostic term) or secondary to other known disease entities. Secondary MPGN is classified into 2 broad categories based on immunofluorescence staining pattern (in corroboration with molecular testing for alternative complement pathway regulatory proteins). These are the following: (i) C3 glomerulopathy (which includes C3 GN and dense deposit disease) that has C3-dominant glomerular staining with little or no Igs or classical complement pathway components such as C1q and C4; (ii) immune complex MPGN with dominant or codominant Ig and C3 staining with or without C1q<sup>1,2</sup>; (iii) chronic thrombotic microangiopathy; and (iv) radiation nephritis. Category 2 includes lupus nephritis, infection-associated GNs, polyclonal cryoglobulinemic GN, and monoclonal gammopathy-associated glomerular diseases, mainly, proliferative GN with monoclonal IgG deposit (PGNMID).

Dominant C3 immunofluorescence staining is considered a defining feature in the biopsy diagnosis of C3GN.<sup>1</sup> Interestingly, however, dominant/co-dominant C3 staining in glomerular deposits can be found even in PGNMID and some immune complex-mediated GNs, most frequent being infection-associated GN (both poststreptococcal GN and active infection-associated GN owing to Staphylococci and other bacteria).<sup>3</sup> Nasr

*et al.*<sup>4</sup> reported up to 97% of their PGNMID cases as revealing C3 staining.

Many of these diseases, particularly C3GN, PGNMID, and lupus nephritis, tend to have a protracted course with repeated disease flares, often requiring repeated biopsies.<sup>5,6</sup> Interestingly, consecutive biopsies from the same patient have on rare occasions been reported to reveal some variation in the immunofluorescence pattern.<sup>1,5</sup> Here, we describe an interesting case of a young woman with a distinct MPGN pattern of injury and a clinical course spanning 14 years without satisfactory response to immunosuppressive treatment, negative autoimmune serology results, requiring 4 kidney biopsies revealing an evolving immunofluorescence pattern making it difficult to accurately classify the glomerular disease according to conventionally defined categories.<sup>6,7</sup> After several changes to her therapeutic regimen, our patient continues to maintain stable renal function with decreased subnephrotic proteinuria. We highlight important teaching points to help tackle such challenging biopsy cases in terms of pathology diagnosis and management approach.

## CASE PRESENTATION

This is a 30-year-old White female with MPGN pattern of GN since 2007. Otherwise active, she first noticed increasing lower extremity edema during early 2007 while traveling. This gradually progressed, and she presented during late 2007 with edema and shortness of breath. Serum creatinine level increased from 0.8 to 1.1 mg/dl in 3 days and proteinuria level from 2.6 to 4 g on 24-hour collection, with low C3 and C4 (details in

Table 1). This prompted her first kidney biopsy in 2007, in which she was diagnosed with having MPGN type I with co-dominant strong (2 to 3+) IgG, lambda, and C3 staining and mild IgM, C1q, and kappa. Subclass staining results revealed dominant IgG3. A few small endothelial tubuloreticular inclusions were also identified (Supplementary Figure S1A–H). She was treated with courses of steroids and azathioprine. The second biopsy was done in 2013 at an outside institution for increasing proteinuria level, in which she was diagnosed with having MPGN type III. The pathology report indicated presence of strong IgG and C3 in the mesangium and capillary loops as before, but also strong IgA, IgM, C1q, kappa, and lambda along the capillary loops (“full-house” staining pattern). No tubuloreticular inclusions were reported. She was again referred to our institution in January 2018 while on azathioprine. She was normotensive, with urine protein-to-creatinine ratio of 1.7, low C3 and C4, normal renal function, and no clinical or serologic features of systemic lupus erythematosus. Serum testing result revealed C3, C4, and C5 nephritic factors. She therefore underwent a third kidney biopsy (Figure 1a–e), which again revealed IgG and C3 codominant staining with IgG3 subclass predominance but no clear light-chain restriction. Because of the IgG3 subclass dominance on biopsy, and the lack of response to her previous immunosuppressive regimen, the possibility of monoclonal gammopathy of renal significance was raised. Treatment with anti-B cell agent rituximab was begun, but without any clinical improvement. Urine protein-to-creatinine ratio increased to 2.6. Her regimen was subsequently changed to antiplasma cell therapy with bortezomib and dexamethasone, which were continued for a duration of 8 months, but urine protein-to-creatinine ratio increased to 3. A complement mutation panel for alternative pathway regulatory proteins was done (Iowa University), and the result was negative. Treatment regimen was changed to daratumumab (anti-CD38 monoclonal antibody) in December 2019. In January 2020, however, she was found to have a monoclonal spike with IgG kappa protein (peaked at 51 mg/dl), with persistent proteinuria (2.4 g/24 h), which was intermittently in the nephrotic range with low serum complement levels with C3 <15 mg/dl and C4 at 11 mg/dl. Nevertheless, the nephritic factors disappeared. The fourth kidney biopsy was performed in late 2020. It revealed a persistent MPGN pattern of GN, but now with weak to absent IgG, IgA, IgM, C1q, kappa, and lambda staining (confirmed with antigen retrieval), but persistent 2 to 3+ C3 (Figure 1f and g). Importantly,

electron microscopy result revealed significantly fewer electron-dense deposits as compared with that found in the 3 previous biopsies. The serum monoclonal IgG kappa spike progressively decreased to 14 mg/dl in a period of 6 months. Furthermore, proteinuria started to decline. Serum C3 levels substantially improved to 97 mg/dl. The patient continues to have a stable serum creatinine level in the range of 0.6 to 0.9 mg/dl. Daratumumab was stopped in July 2021, and the patient continues to be maintained on mycophenolate.

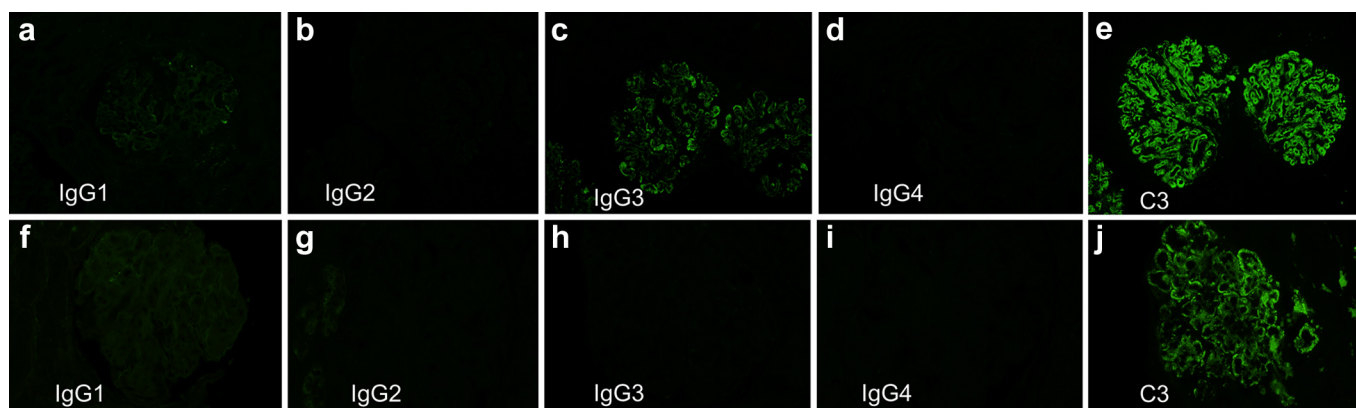
## DISCUSSION

The glomerular disease in this patient was difficult to classify. It had some immunohistologic features of PGNMID, lupus-like immune complex GN, and C3 GN, making it an intriguing “overlap.” The first biopsy was supportive of PGNMID, but the young age of the patient and presence of endothelial tubuloreticular inclusions were quite unusual.<sup>4</sup> In addition, at that time, this entity of PGNMID was not well described, and therefore, a diagnosis of MPGN type I was made. Age incidence of PGNMID is higher in the older population and C3GN in the pediatric and adolescent populations,<sup>1,2,4</sup> but occasionally each of these diseases can occur in patients at the other end of the age spectrum.<sup>6,53</sup> The full-house pattern found in the second biopsy was reminiscent of lupus nephritis, but there were no supportive clinical features or serologic results. The third biopsy again revealed IgG3 subclass dominance with strong C3 raising the possibility of PGNMID, but no definite light-chain restriction was evident. Furthermore, other Ig heavy chains were present (albeit not as strong as IgG). Results of serum immunofixation studies were negative, but that is not unusual in PGNMID.<sup>4</sup> The fourth (last) biopsy revealed persistent C3 staining with absence of IgG. Considering the persistently low serum C3 level and transient C3, C4, and C5 nephritic factors, this IF pattern was reminiscent of C3GN.<sup>1,2</sup> Complement-stabilizing autoantibodies (nephritic factors) are not typically encountered (or rarely tested for) in PGNMID but have been reported.<sup>53</sup> Paraproteins however can have nephritic factor activity. Conversely, C3GN has been described in the background of monoclonal gammopathy in elderly patients (and included under the category of monoclonal gammopathy of renal significance).<sup>6,7</sup> In our patient, the nephritic factors subsequently disappeared with anti-CD38 (daratumumab) treatment, but to further complicate the picture, new serum IgG kappa monoclonal spike appeared after treatment with daratumumab was begun. Humanized monoclonal antibodies used in the treatment (such as eculizumab and

**Table 1.** Consecutive renal biopsy and laboratory test results

Year of biopsy	2007 (Biopsy 1 at our institution)	2013 (Biopsy 2 at outside institution)	2017	2018 (Biopsy 3 at our institution)	2019	2020 (Biopsy 4 at our institution)	2021
Biopsy diagnosis	MPGN type I	MPGN type III	Work-up for alternate complement pathway mutations negative	PGNMID with dominant C3 staining	Trial of rituximab, Velcade, but persistent proteinuria. Addition of daratumumab.	MPGN with persistent C3 staining	Daratumumab stopped; MMF started
Light microscopy and DIF findings	MPGN pattern with no IF/TA. 2 to 3+ IgG, 3+ C3 staining, 1+ C1q, IgM, kappa and 2 to 3+ lambda granular mesangial and glomerular capillary wall staining.	MPGN pattern with 10% IF/TA. 2 to 3+ IgG, and 3+ C3 granular mesangial and glomerular capillary wall staining. 2+ IgA, IgM, C1q, kappa and lambda capillary wall.		MPGN pattern with <10% IF/TA. 1 to 2+ IgG, 3+ C3 granular mesangial and glomerular capillary wall, 1+ IgA, 1+ IgM, trace C1q and 1+ kappa and 1+ lambda	Detection of IgG kappa spike in serum 51 mg/dl, but subsequently decreased to 14 mg/dl in 5 mo. Serum-free kappa: lambda free light-chain ratio also revealed a progressive decline	MPGN pattern with <10% IF/TA. 2 to 3+ C3 granular mesangial and glomerular capillary wall, trace IgG, IgM, C1q, negative IgA, and negative kappa and lambda. Antigen retrieval with proteinase digestion also negative for IgG, kappa or lambda staining.	
IgG subclass DIF	3+ IgG3, other subclasses negative	Not performed		2+ IgG3, other subclasses negative		Negative for all IgG subclasses	
Electron microscopy	Numerous mesangial, subendothelial, intramembranous, and subepithelial immune-type deposits, rare small endothelial TRIs, widespread foot process effacement	Numerous mesangial, subendothelial, intramembranous, and subepithelial immune-type deposits, widespread foot process effacement		Numerous immune-type deposits, large subepithelial deposits, numerous subendothelial and mesangium, and few intramembranous, no TRIs, prominent foot process effacement		Deposits fewer than previous biopsies. Few discrete subepithelial deposits. Others partially resorbed with electron lucencies. GBM showed widespread subendothelial widening, GBM duplication, mesangial cell interposition, no TRIs	
Serum C3; C4	<6; 11 mg/dl	Not available	<15; 12	<15; 14	<15; 18	66; 18	86; 24
Urine protein	4 g/24 h	Not available	1.74 UPC ratio	0.8 UPC ratio	3 g/24 h	2.4 g/24 h	1.8 g/24 h
S. Cr.	1.1 mg/dl	Not available	0.8	0.6	Not available	0.6 to 0.9	0.6
Monoclonal work-up	SPEP, Sfix negative	Not repeated	Not repeated	Bone marrow biopsy negative for plasma cell dyscrasia. Sfix negative	Sfix negative	Sfix negative	Sfix negative
Other	n/a	n/a	n/a	C3, C4, and C5 nephritic factors detected	No testing done	Disappearance of the nephritic factors	n/a

DIF, direct immunofluorescence; GN, glomerulonephritis; IF/TA, interstitial fibrosis and tubular atrophy; MMF, mycophenolate; MPGN, membranoproliferative glomerulonephritis; n/a, not applicable; PGNMID, proliferative GN with monoclonal IgG deposit; S. Cr., serum creatinine; Sfix, serum immunofixation; SPEP, serum protein electrophoresis; TRI, tubuloreticular inclusion; UPC, urine protein-to-creatinine ratio.



**Figure 1.** IgG subclass and C3 immunofluorescence staining in biopsy no. 3 (a–e) revealing 2+ or moderate glomerular staining for IgG3 and 3+ C3 and negative staining for IgG1, IgG2, and IgG4. Biopsy no. 4 (f–j) reveals persistent 3+ glomerular C3 staining and negative for the IgG subclasses.

daratumumab) have been found to persist in tissue and/or in the serum giving false positive results on tissue immunostaining or serum immunofixation assays.<sup>8,S4,S5</sup> The levels slowly diminished; however, this finding can be misleading if the pathologist is not aware of the newer evolving therapeutic modalities and their pitfalls.

Overall, this could have been a case of PGNMID from the outset in a young patient, with a very insidious course and persistent nephrotoxic plasma cell clone. The “full-house” IF staining could be an artifact (staining with other heavy chains was described only in the peripheral capillary loops). The plasma cell clone remained active despite immunosuppressive therapy but did reveal suppression after beginning the anti-plasma cell therapies, the most effective being daratumumab. The IgG staining disappeared in the last biopsy, and deposits on EM were less. Why the C3 staining persisted remains unclear.

PGNMID and C3GN can have histologic similarities—MPGN pattern of glomerular injury, granular non-organized deposits on electron microscopy, and strong C3 staining.<sup>1,2</sup> C1q staining is thought to be more frequent in PGNMID, but not totally absent in C3GN.

We analyzed the cohort of PGNMID ( $n = 45$ ) and C3GN ( $n = 16$ ) cases at our institution received during an 8-year period from 2011 to 2019 (Table 2). Median patient age in our PGNMID cohort was much higher (56 years) than in C3GN (20 years), but the age range was quite broad in both diseases (12 to 89 in PGNMID and 14 to 73 in C3GN). C3 staining was very frequently found in PGNMID (all except 1 case) along with IgG,

with 20 biopsy results revealing equal intensity for IgG and C3, 9 revealing C3 stronger than IgG, and 16 revealing IgG stronger than C3 (difference usually only 1 grade level). On a scale of 0 to 3, median staining intensity for IgG was 2.3 and that for C3 was 2.1. Data for serum C3 and C4 levels were not consistently available, hence not revealed, but Nasr *et al.*<sup>4</sup> have reported low serum C3 levels in up to 8.1% of their patients with PGNMID.<sup>3</sup> Importantly, C1q staining was more frequent in PGNMID (60% biopsies), but it can be found in C3GN, albeit less frequently (31% biopsies) and in weaker intensity (Table 2).

Even though these diseases are construed as separate entities, recent work suggests that complement dysregulation lies at the core, and as elegantly reported by Iatropoulos *et al.*,<sup>9</sup> these groups of diseases may in fact represent clusters along a spectrum of complement dysregulation. Differences may lie in the triggering events, patient age, and contribution of classical versus alternative (or other) pathways of complement activation. Time and again, intriguing similarities between these disease clusters come to light in the form of overlapping biopsy features, or even fortuitous response to unexpected therapeutic drugs.<sup>5,S3,S6</sup> An example of the latter was found in a 9-year-old male with a protracted course of C3GN who ultimately responded favorably to plasma cell therapy with bortezomib, but not with C5 blocker (eculizumab) and other immunosuppressives.<sup>S6</sup> Plasma cell antagonist bortezomib is a treatment modality typically used for treating monoclonal gammopathy-related kidney disease (such as PGNMID), but in this case, it unexpectedly had favorable response in C3GN. The mechanism

**Table 2.** Basic demographic features and immunofluorescence staining intensity in our cohort of PGNMID and C3GN in an 8-year period

	Median age in yr (range)	Sex male/female	Mean IgG	Mean C3	Mean C1q	No. of cases with C1q
PGNMID ( $n = 45$ )	56 (12–89)	23 M/22 F	2.3	2.1	1	27/45 (60%)
C3GN ( $n = 16$ )	22 (14–73)	10 M/6 F	0.75	3	0.5	5/16 (31%)

F, female; GN, glomerulonephritis; M, male; PGNMID, proliferative GN with monoclonal IgG deposit.

**Table 3.** Teaching points

1. The MPGN pattern on kidney biopsy can be associated with a spectrum of disease entities, ranging from immune complex GN to complement-mediated GN, and monoclonal gammopathy-associated kidney diseases, particularly PGNMID. In occasional cases, histologic findings can be overlapping. In addition, evolving IF pattern in consecutive biopsies may be found, making diagnosis difficult. In such cases, it may be preferable to keep the pathology report descriptive rather than trying to "fit" the case into a conventionally accepted classification. It is more important to meticulously integrate the findings from the previous biopsies along with serologic results and tailor the treatment strategies accordingly. Molecular testing for alternative complement pathway regulatory proteins and nephritic factors is helpful.
2. IgG subclass staining is strongly recommended in such cases. Monotypic IgG staining is a useful finding when kappa and lambda light-chain staining results are ambiguous (or if the latter are not routinely performed as in pediatric kidney biopsies). IF staining with pronase digestion in biopsies with strong C3 alone is also important to confirm or exclude "masked" IgG.
3. Monoclonal humanized antibodies used in the treatment can themselves be detected in laboratory assays and on tissue staining. Awareness of this pitfall is important for the renal pathologist and the treating nephrologist to avoid confusion and overdiagnosis.

IF, immunofluorescence; GN, glomerulonephritis; MPGN, membranoproliferative glomerulonephritis; PGNMID, proliferative GN with monoclonal IgG deposit.

of action could be through suppressing the production of autoantibodies by plasma cells (the C3 nephritic factors).

Nephrotoxic plasma cell clones and secreted paraproteins (even undetectable by our testing methods) seem to be important triggers mediating complement-induced glomerular injury, even in young patients. Monotypic IgG subclass staining if present can be an important clue in such cases even when definitive light-chain restriction is not evident in the biopsy. Although not a complete disease cure, the antiplasma cell therapy with daratumumab did help to maintain normal renal function, keep the proteinuria in check, and noticeably reduce the degree of immune complex deposits in our patient. The teaching points gleaned from this case are provided in point-by-point format (Table 3).

## DISCLOSURE

All the authors declared no competing interests.

## PATIENT CONSENT

The authors declare that they have obtained consent from the patient discussed in the report.

## SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

**Figure S1.** Immunofluorescence stain results from biopsy no. 1. (A) IgG: 2 to 3+ diffuse granular mesangial and glomerular capillary staining; (B) C3: 3+; (C) trace kappa; (D) lambda: 2 to 3+; (E) IgG1 trace; (F) IgG2 negative; (G) 2 to 3+ IgG3; and (H) IgG4 negative.

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