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Original Article

Reanalysis of membranoproliferative glomerulonephritis patients according to the new classification: a multicenter study [☆]



Sung Ae Woo ¹, Hye Young Ju ², Soon Hyo Kwon ¹, Ji-Hye Lee ³, Soo Jeong Choi ²,
 Dong Cheol Han ¹, Seung Duk Hwang ², Sae-Yong Hong ⁴, So-Young Jin ⁵, Hyo-Wook Gil ^{4,*}

¹ Department of Internal Medicine, Soonchunhyang University Seoul Hospital, Seoul, Korea

² Department of Internal Medicine, Soonchunhyang University Bucheon Hospital, Bucheon, Korea

³ Department of Pathology, Soonchunhyang University Cheonan Hospital, Cheonan, Korea

⁴ Department of Internal Medicine, Soonchunhyang University Cheonan Hospital, Cheonan, Korea

⁵ Department of Pathology, Soonchunhyang University Seoul Hospital, Seoul, Korea

ABSTRACT

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Background: All types of membranoproliferative glomerulonephritis (MPGN) are progressive diseases with poor prognoses. Recently, a newly proposed classification of these diseases separated them into immune complex- and complement-mediated diseases. We investigated the frequency of C3 glomerulonephritis among previously diagnosed MPGN patients.

Methods: We conducted a retrospective study of patients diagnosed with MPGN at three tertiary care institutions between 2001 and 2010. We investigated the incidence of complement-mediated disease among patients diagnosed with MPGN. Progressive renal dysfunction was defined as a 50% reduction in the glomerular filtration rate or the need for renal replacement therapy.

Results: Among the 3,294 renal biopsy patients, 77 (2.3%) were diagnosed with MPGN; 31 cases were excluded, of which seven were diagnosed with systemic lupus nephritis, and the others were not followed for a minimum of 12 months after biopsy. Based on the new classification, complement-mediated MPGN was diagnosed in two patients (4.3%); only one patient developed progressive renal dysfunction. Among the immune complex-mediated MPGN patients, 17 patients developed progressive renal dysfunction. Serum albumin and creatinine levels at the time of MPGN diagnosis were risk factors of renal deterioration, after adjusting for low C3 levels and nephrotic syndrome.

Conclusion: Complement-mediated glomerulonephritis was present in 4.3% of patients previously diagnosed with MPGN.

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Introduction

Membranoproliferative glomerulonephritis (MPGN) describes a general pattern of glomerular injury seen in a variety of disease processes sharing a common pathogenic mechanism rather than a single disease entity. Primary MPGN was previously classified according to its histopathological pattern (types I, II, and III), based on the ultrastructural location of electron dense deposits.

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* Corresponding author. Department of Internal Medicine, Soonchunhyang University Cheonan Hospital, 31 Soonchunhyang 6 gil, Dongnam-gu, Cheonan, Chungnam 330-721, Korea.

E-mail address: hwgil@schmc.ac.kr (H-W Gil).

An improved understanding of the role of complement in the pathogenesis of MPGN has led to a paradigm shift in the disease classification. The proposed reclassification of MPGN into immunoglobulin-mediated disease via activation of the classical complement pathway, versus nonimmunoglobulin-mediated disease via activation of the alternative complement pathway (AP), has led to improved diagnostic algorithms and the emergence of a new disease category called C3 glomerulonephritis (C3GN) [1]. Improved understanding of MPGN, particularly the focus on the activity of the AP in C3GN, will lead to improved disease outcomes [2]. However, the frequency of C3GN remains unknown among patients previously diagnosed with MPGN. MPGN patients typically demonstrate poor renal outcomes, with a reported 10-year renal survival rate of approximately 40%. Prognoses according to the new classification, however, are unknown; studies on two cohorts have shown that C3GN is not a benign disease [3,4].

We conducted a multicenter reanalysis of a 10-year renal biopsy registry, to investigate the incidence and long-term outcomes of C3GN among MPGN patients.

Methods

Patients

We collected retrospective clinical data on patients diagnosed with MPGN, based on renal biopsy findings at three tertiary care institutions (Soonchunhyang University, Seoul, Korea, Bucheon Hospital, Bucheon, Korea, and Cheonan Hospital, Cheonan, Korea), between 2001 and 2010. Patients who were followed for > 12 months were included in the study. Patients diagnosed with lupus nephritis were excluded.

We investigated the following results (collected at the time of diagnosis): complete blood counts; renal function tests; liver function tests; urine analyses; and levels of serum C3, serum C4, immunoglobulin G, serum albumin, serum hepatitis B surface antigens (HBsAg), anti-hepatitis B surface antibody, and anti-hepatitis C antibody (HCV Ab). All renal biopsy specimens were examined by a pathologist using light, immunofluorescence, and electron microscopy at the time of the initial biopsy. We reviewed the pathologic findings and reclassified the patients as having immunoglobulin-mediated MPGN when immunoglobulin and complement were evident on immunofluorescent microscopy or as having complement-mediated MPGN when marked immunoglobulin staining was not observed by immunofluorescent microscopy.

The diagnosis of type I or III MPGN was based on the presence of a kidney disorder characterized by mesangial cell proliferation and structural changes in the glomerular capillary walls [5]. C3 glomerulonephritis was defined by the presence of glomerular C3 staining, in the absence of immunoglobulin, together with electron-dense subendothelial glomerular basement membranes and mesangial deposits [4,5].

Hypertension was diagnosed if the patient had a systolic blood pressure (BP) of ≥ 140 mmHg and/or diastolic BP of ≥ 90 mmHg or was prescribed antihypertensive medication(s). Microscopic hematuria was defined as ≥ 3 red blood cells/high power field. Nephrotic syndrome was defined based on the presence of proteinuria (≥ 3.5 g/d) and hypoalbuminemia (serum albumin < 3.0 g/dL). The glomerular filtration rate (GFR) was estimated using the Chronic Kidney Disease Epidemiology Collaboration equation [6]. Renal impairment at the time of the biopsy was defined as a GFR of < 60 mL/min at

Table 1. Patient characteristics at the time of renal biopsy

Characteristics	
Age (y)	45.9 (18–84)
Sex (M/F)	28/18
LM classification	
Type I/type III	28/18 (60.8/39.1)
IF classification	
Immune mediated/complement mediated	44/2 (95.7/4.3)
HBs antigenemia	17 (37.7)
Hypertension	19 (41)
Creatinine (mg/dL)	1.25 \pm 0.95
GFR (mL/min)	61.24 \pm 41.89
Hematuria*	38 (84.4)
Urine protein (mg/24 h)	4,644.58 \pm 4,521.98
Nephrotic syndrome [†]	21 (45.6)
Albumin (g/dL)	2.94 \pm 0.78
Decreased C3 [‡]	13 (28)

* ≥ 3 red blood cells/high power field.

[†] Proteinuria ≥ 3.5 g/day and serum albumin < 3.0 g/dL.

[‡] Serum C3 < 65 mg/dL.

Data are presented as *n* (%) or mean \pm SD, unless otherwise indicated. F, female; GFR, glomerular filtration rate; HBs, hepatitis B surface antibody; IF, immunofluorescence; LM, light microscopy; M, male; SD, standard deviation.

diagnosis. Progressive renal dysfunction was defined as a 50% reduction in the estimated GFR (eGFR) or the need for renal replacement therapy during the follow-up period. Stable renal function was defined as renal function maintained at $< 50\%$ reduction in eGFR during the follow-up period.

Statistical analysis

Statistical analyses were performed with SPSS version 14.0 (SPSS Inc., Chicago, IL, USA). Data were expressed as mean \pm standard deviation. Results yielding $P < 0.05$ were considered statistically significant. Significant differences between groups were determined using independent *t* tests. Categorical variables were compared using either the Pearson's Chi-square test or Fisher's exact test. Multiple logistic regression analysis was performed to identify the independent risk factors for renal death among the parameters selected through univariate analysis.

Results

Among the 3,294 patients undergoing renal biopsy during the study period, 77 (2.3%) were diagnosed with MPGN. Thirty-one cases were excluded because seven patients were diagnosed with systemic lupus erythematosus, and the others were not followed up for the required 12 months after biopsy; thus, 46 cases were eligible for analysis. Lupus nephritis was excluded because the prognosis was different. The clinical manifestations, at the time of the renal biopsy, are shown in Table 1. For the 46 included patients (males, 28; females, 18), the mean age was 45 years and the mean eGFR was 61.2 \pm 41.9 mL/min. Thirty-eight patients (84.4%) had hematuria, and 20 patients (45.6%) had nephrotic syndrome. Over a mean follow-up period of 82.8 months, 17 patients (39.6%) developed progressive renal dysfunction, of which nine patients (19.6%) required renal replacement therapy.

According to the classical pathologic classification, 28 patients (60.8%) were classified with MPGN type I, and 18 patients (39.2%) were classified with MPGN type III. Following the new classification, immune-mediated MPGN was observed

Table 2. Clinicolaboratory findings between patients with stable renal function and those with progressive renal dysfunction

Characteristics	Stable renal function (n=28)	Progressive renal dysfunction (n=16)	P
Age (y)	44.6 (23–72)	47.9 (18–84)	0.547
Sex (M/F)	15/13	13/3	0.066
HBs antigenemia	9 (32)	8 (50)	0.280
Systolic blood pressure (mmHg)	131.43 ± 20.31	140.33 ± 22.56	0.213
Diastolic blood pressure (mmHg)	80.71 ± 9.79	90.00 ± 14.64	0.017
Creatinine (mg/dL)	0.94 ± 0.41	1.82 ± 1.37	0.023
Glomerular filtration rate	97.7 ± 37.65	61.33 ± 33.04	0.003
Hematuria*	24 (85.7)	13 (86.7)	0.932
Urine protein (mg/24 h)	4,332.25 ± 4,391.90	5,545.44 ± 4,926.23	0.404
Nephrotic syndrome	11 (39.3)	9 (56.3)	0.227
Albumin (g/dL)	3.17 ± 0.73	2.47 ± 0.63	0.003
C3 (mg/dL)†	81.98 ± 29.40	62.08 ± 24.04	0.029
C4 (mg/dL)‡	19.81 ± 9.27	21.26 ± 13.02	0.680
CH ₅₀ (U/mL)§	27.73 ± 10.78	23.73 ± 9.91	0.528
IgG (mg/dL)¶	875.87 ± 298.39	1,070.89 ± 973.66	0.478

* ≥ 3 red blood cells/high power field.

† C3 reference range, 65–135 mg/dL.

‡ C4 reference range, 13–35 mg/dL.

§ CH₅₀ (50% hemolytic unit of complement reference range, 23–46).

¶ IgG reference range, 870–1,700 mg/dL.

Data are presented as n (%) or mean ± SD, unless otherwise indicated.

F, female; HBs, hepatitis B surface antibody; IgG, immunoglobulin G; M, male; SD, standard deviation.

in 39 patients (95.7%); only two patients (4.3%) were classified with complement-mediated MPGN, both of whom had C3GN. One C3GN patient developed end-stage renal disease within 23 months from the time of biopsy. Renal impairment (eGFR, 41 mL/min), hypoalbuminemia (2.4 g/dL), nephrotic syndrome, and hypertension were observed at the time of the biopsy in this case. The other C3GN patient maintained renal function during the follow-up period (41 months). This individual displayed normal kidney function (eGFR, 99 mL/min), normal BP, and mild proteinuria (urine protein, 471 mg/24 h) at the time of the biopsy.

According to the classic pathologic classification, among the patients with immune complex-mediated MPGN, 27 (61.4%) had MPGN type I and 17 (38.6%) had MPGN type III. Of the 27 type I and 17 type III patients, 11 (41%) and five (29%), respectively, developed progressive renal dysfunction. However, there was no significant difference in the incidence of progressive renal dysfunction between the two types ($P=0.447$). Among the patients with immune complex mediated MPGN, 15 had positive HBsAg, one had HBsAg positive with HCV Ab positive, and one had cancer at diagnosis.

We additionally divided the patients with immune complex-mediated MPGN into those with stable renal function and those with progressive renal dysfunction. The clinical and laboratory parameters of the two groups are compared in Table 2. Renal function, at diagnosis, was impaired to a greater extent in patients with progressive renal dysfunction (serum creatinine, $P=0.023$; eGFR, $P=0.003$), and they had significantly lower serum albumin and C3 levels, than those in the stable renal function group. There were no significant differences in hepatitis B surface antibody levels, antigenemia, hematuria, or proteinuria between the two groups. We also analyzed the prognostic factors that influenced renal outcome. According to the univariate analysis, serum albumin and creatinine levels at diagnosis, and GFR were significant risk factors, whereas sex, hypertension, nephrotic syndrome, low C3 levels, and age were not. Serum albumin and creatinine levels, at diagnosis, remained significant risk factors after adjusting for complement levels and the presence of nephrotic syndrome (Table 3).

Table 3. Possible predictive risk factors of progressive renal dysfunction

	Odds ratio	95% confidence interval	P
Nephrotic syndrome	0.293	0.040–2.121	0.224
Serum albumin	0.170	0.037–0.775	0.022
Serum creatinine	9.161	1.024–81.920	0.048
Low complement 3 (C3 < 65 mg/dL)	2.665	0.427–16.627	0.294

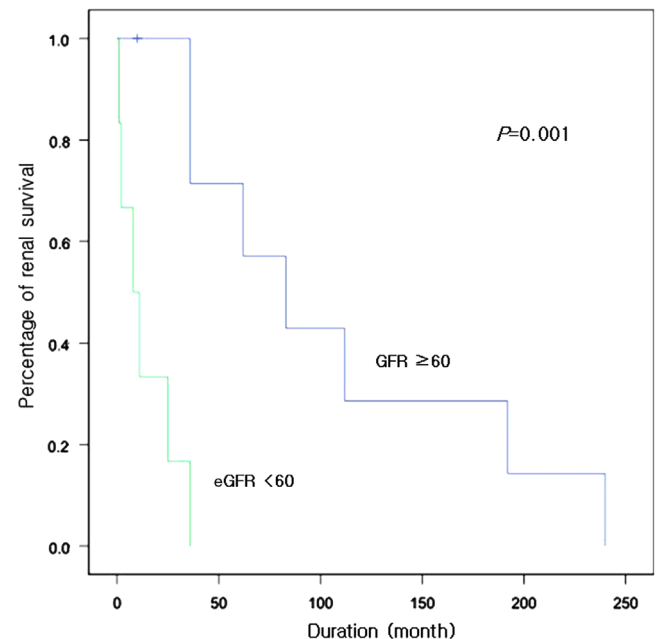


Figure 1. The renal survival curve during long-term follow-up. The initial glomerulofiltration rate is an important prognostic factor for membranoproliferative glomerulonephritis patients (Kaplan–Meier).

Renal survival rate was decreased in patients with renal impairment at the time of the biopsy, compared with those without renal impairment ($P=0.001$; Fig. 1). All patients

received renin–angiotensin system inhibitors, and 16 patients were also treated with immunosuppressants. Among those treated with immunosuppressants, five patients experienced an overall 50% reduction in eGFR. Six patients died during the follow-up period owing to cancer (4 patients) or hepatorenal syndrome with hepatocellular carcinoma or liver cirrhosis (2 patients). Cancer was diagnosed in six patients; one patient had stomach cancer at the time of renal biopsy, and the others developed cancer (stomach cancer, 1 patient; rectal cancer, 1 patient; lung cancer, 1 patient; hepatocellular carcinoma, 2 patients) during the follow-up period.

Discussion

Our study showed that the incidence of C3GN was 4.3% among patients with previously diagnosed MPGN who were followed up for > 12 months. The incidence of C3GN among MPGN patients has not been reported previously. MPGN is a rare disease that leads to an extremely rare incidence of C3GN.

The concept of C3GN was first introduced in 2007 when Servais et al [7] characterized a group of patients with isolated C3 deposits, previously referred to as proliferative glomerulonephritis with C3 deposits. Some glomerulonephritides may appear because of dysregulation of the AP. Mutations or antibodies against multiple complement regulators and inhibitors that prevent self-mediated damage can alter the control of the AP and lead to the development of MPGN [1,7,8]. C3GN is characterized by prominent glomerular C3 deposition, without significant immunoglobulin deposits evident on immunofluorescent microscopy. Complement proteins from the AP and terminal complement complexes, as well as complement regulating proteins, were found in the glomeruli of C3GN patients. Proteins of the classic pathway, such as C1 and C4, were not present in patients with C3GN [3].

A better understanding of the disease pathogenesis has led to newly proposed assessment and treatment methods. Recently, newer drugs, such as the anti-C5 monoclonal antibody, eculizumab, have been suggested for treating complement-mediated MPGN [9–11]. Bomback et al [12] reported that eculizumab improved the clinical and histopathologic findings in C3GN patients. Three patients with C3GN were treated with eculizumab for 53 weeks. Some patients showed improved renal function, and normalization of elevated levels of soluble membrane attack complexes. Conversely, some patients showed declining renal function during treatment [12]. Elevation of soluble membrane attack complex (C5b-9) levels, an indicator of the activation of the terminal complement cascade, is predictive of a response to eculizumab treatment [10,12]. The patient in our study had poor prognostic factors, including renal impairment, and showed poor renal outcomes. During the follow-up period, the patient received only renin–angiotensin system blockers and progressed to end-stage renal disease. The effectiveness of nonspecific immunomodulatory therapy is controversial [13,14]. Therefore, eculizumab may be a treatment option for C3GN patients with poor prognostic factors.

The disease prognosis of the recently described C3GN remains largely unknown. Very few studies have described the clinical features of C3GN. Two C3GN patients in one study maintained their renal function [8]. Another study described the renal outcomes of 12 patients with C3GN, including two patients undergoing allograft transplantation. Among the 12

patients, two developed progressive renal dysfunction (50% reduction of eGFR or the need for renal replacement therapy) and both developed recurrent disease following allograft transplantation [4]. C3GN had a very low prevalence (4.3%) among the MPGN patients in our study. Complement analysis was not performed, because the pathogenesis of C3GN was unknown at the time of biopsy. The number of patients with C3GN was too small to represent the overall renal outcomes for patients with the disease. One of the two C3GN patients, in this study, progressed to renal dysfunction. This patient had high classical risk factors. Further study should reveal the prognostic factors related to disease pathogenesis.

Classical signs of nephrotic syndrome, elevated serum creatinine levels, hypertension, and crescents on renal biopsy are indicative of poor prognosis upon presentation with MPGN [15–17]. Our data were very similar to those described in previous studies. Future studies are required to identify the different prognostic factors relevant to both immune-mediated MPGN and C3GN. Further studies should also clarify the features and prognosis factors in immune-mediated MPGN excluding C3GN, because previous studies did not exclude C3GN among the MPGN population.

Our study has several limitations. First, this study was retrospective. Second, we did not evaluate the abnormalities of the alternative pathway because these laboratory data were not routinely investigated. Third, only a very small number of C3GN patients were available for inclusion; hence, the prognostic factors were not fully evaluated. However, our study showed the incidence of C3GN and the individual factors involved in the prognosis of C3GN.

In conclusion, C3GN occurred at a rate of 4.3% among patients previously diagnosed with MPGN. Initial GFR was an important prognosis factor in immune-mediated MPGN. Further studies involving more patients and longer follow-up periods are necessary to conclusively determine the disease prognosis and the usefulness of anticomplement therapy in C3GN.

Conflict of interest

The authors declare no conflict of interest.

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