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Original Article

Clinicopathological analysis of membranous nephropathy with crescents

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Abstract: Objective: To observe the clinicopathological characteristics of patients with membranous nephropathy (MN) and crescents. Methods: Fifty-eight patients with biopsy-proven MN and crescents, in the absence of immunologic and clinical etiologic factors, were enrolled in this retrospective study. Another 100 MN patients without histological crescents were used as a control group. The clinicopathological features, treatment response and outcome were analyzed and compared between groups. Results: The 58 patients with crescents accounted for 1.4% of a total of 4,200 patients with biopsy-proven MN. The mean age was 50.2±14.3 years. The rates of hypertension (51.7% vs 19.0%, P=0.000) and decreased eGFR (25.9% vs 4.0%, P=0.000) were higher than those in the control group. Circulating autoantibodies against M-type phospholipase A_2 receptor (PLA_2R) were found in 32 of 50 (64.0%) patients. Glomeruli showed on average 4.6% (range, 1.8%-35.3%) involvement of crescents. Lesions of segmental glomerulosclerosis (48.3% vs 16.0%, P=0.000), capillary loops necrosis (10.3% vs 0.0%, P=0.002), interstitial fibrosis/tubular atrophy (IFTA) (87.9% vs 54.0%, P=0.000) and afferent arterial lesions (89.7% vs 65.0%, P=0.001) were more common. The outcomes of the patients with crescentic MN did not differ from those in the control group. Conclusions: MN with crescents is rare, and secondary MN and crescentic glomerulonephritis should be considered. Crescentic MN usually presents with hypertension and renal dysfunction clinically and with, severe segmental and global glomerulosclerosis, capillary loops necrosis and IFTA histologically, and the condition has a favorable prognosis.

Keywords: Membranous nephropathy, crescent, clinical pathology

Introduction

Membranous nephropathy (MN) is a common cause of nephrotic syndrome in adults and can be primary or secondary to systemic lupus erythematosus (SLE), chronic infection, tumors, or drugs. Endocapillary proliferation, necrosis, and crescents are rare in idiopathic membranous nephropathy (IMN), and always suggest secondary MN or MN superimposed on anti-glomerular basement membrane (anti-GBM) nephritis [1-4], systemic vasculitis [5, 6], or IgA nephropathy [7]. Some patients with crescentic MN exhibit no evidence of secondary MN, anti-GBM nephritis, systemic vasculitis, or IgA nephropathy but contain circulating antibodies directed against PLA_2R [8]. In the present study, we report a series of such patients and examine their clinicopathological features and outcomes.

Materials and methods

Study participants

Patients with crescentic IMN from the Institute of Kidney Disease of the Chinese People's Liberation Army, Jingling Hospital, Nanjing, China, were recruited for the study from April 2005 to April 2015. The study received institutional review board approval. The inclusion criteria were as follows: (i) patients with biopsy-proven MN and crescents; (ii) patients without a history of autoimmune diseases; (iii) patients without a history of viral hepatitis; (iv) patients without a history of tumors; (v) patients with no history of exposure to mercury, formaldehyde or volatile hydrocarbons; and (vi) patients who had not received treatment with gold compounds, penicillamine, rifampicin, bucillamine, lithium compounds or contraceptives. Another 100 IMN

Table 1. Clinical and laboratory features of renal damage

	MN with crescents group	Control group	*P* value
Age, years	50.2±14.3	40.4±14.3	0.000
Males, n (%)	37 (63.8)	58 (58.0)	0.504
Peripheral edema, n (%)	52 (89.7)	85 (85.0)	0.473
Hypertension, n (%)	30 (51.7)	19 (19.0)	0.000
Decreased eGFR, n (%)	15 (25.9)	4 (4.0)	0.000
Negative ANA, n (%)	55 (94.8)	94 (94.0)	1.000
Serum creatinine, mg/dl	0.9 (0.4-6.0)	0.8 (0.4-1.8)	0.000
eGFR, ml/min/1.73 m^2	91.5 (7.2-131.6)	107.2 (44.2-159.3)	0.000
Serum albumin, g/l	31.4±6.2	30.1±6.4	0.231
Urinary protein, g/24 h	4.3 (0.7-22.3)	2.8 (0.5-15.0)	0.360
Nephropathic proteinuria, n (%)	33 (56.9)	47 (47.0)	0.251
Urinary sediment, $\times 10^4$/ml	12.5 (1.0-364.0)	11.5 (1.0-440.0)	0.958

patients without crescent formation were used as a control group. The clinicopathological features and outcomes were compared between the two groups.

Clinical and laboratory data

Gender, age, extrarenal presentations, hypertension, peripheral edema, and decreased eGFR were recorded. The estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology (CKD-EPI) Study equation. Decreased eGFR was defined as <60 ml/min/1.73 m^2. Nephropathic proteinuria was defined as urine protein >3.5 g/24 hours. Serum creatinine and albumin were recorded. Circulating autoantibodies against PLA_2R, antinuclear antibody (ANA), antineutrophil cytoplasmic antibody (ANCA), and anti-GBM were also recorded.

Renal pathology

Ultrasound-guided percutaneous renal biopsy was performed on all participants. For light microscopy examination, the renal biopsy tissues were embedded in paraffin, cut into 1.5-μm sections and then stained with hematoxylin-eosin, periodic acid-Schiff, Masson's trichrome and periodic acid-Schiff-methenamine silver. For immunofluorescence, 4-mmcryostat sections were stained with polyclonal fluorescein isothiocyanate (FITC)-conjugated antibodies to IgG, IgA, IgM, C3, C4, C1q and fibrinogen as per routine clinical testing. The IgG subclasses were evaluated via indirect IF with monoclonal antibodies directed toward IgG1, IgG2, IgG3 and IgG4 in some patients. Some biopsies were stained for PLA_2R via immunofluorescence using a rabbit anti-PLA_2R1 primary antibody (Atlas Antibodies, Stockholm, Sweden) and polyclonal goat anti-rabbit IgG (Life Technologies, Carlsbad, Calif., USA) as the secondary antibody.

Statistical analysis

Statistical analyses were carried out using the SPSS statistical package, version 19.0 (SPSS Inc., Chicago, IL, USA) for Windows. Quantitative data are presented as the means ± SD. Student's t-test for two independent samples was performed for comparisons between two groups. Non-normal distribution data were expressed as the median (range) and analyzed with Mann-Whitney, Kruskal-Wallis, and Wilcoxon tests. Categorical data are presented as the rate (percentage) and were compared using a χ^2 test or Fisher's exact test. A *P*-value <0.05 was considered to be statistically significant.

Results

Proportion

From a total of 4,200 native kidney biopsies showing MN, 75 patients (1.8%) had crescentic MN. Of these, 3 patients had anti-GBM nephritis, 7 patients had ANCA-positive vasculitis, 2 patients had ANCA-negative systemic vasculitis, and 5 patients had IgA nephropathy. The other 58 patients (1.4%) had negative ANCA and negative anti-GBM antibodies.

Clinical and laboratory features

The present research enrolled 37 men and 21 women with an average age of onset of 50.2±14.3 years. No extra renal presentation was observed in the 58 MN patients or 100 patients of the control group. The proportions of hypertension (51.7% vs 19.0%, P=0.000) and decreased eGFR (25.9% vs 4.0%, P=0.000) were higher than those in the control group.

There was no difference in the rates of negative ANA between the two groups (93.0% vs 94.0%,

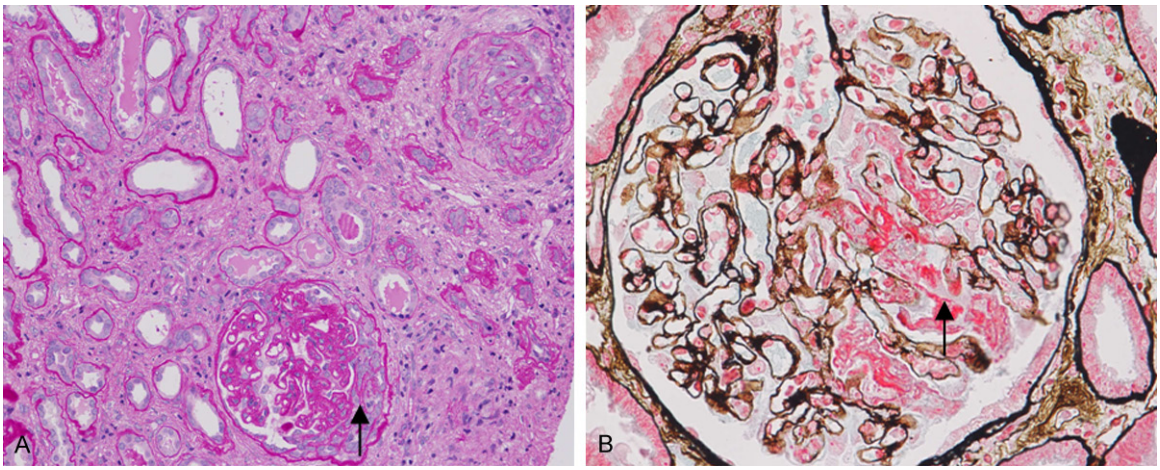

Figure 1. Representative light microscopy of membranous nephropathy and crescents. A. Focal glomerulus shows a cellular crescent (arrow; PAS stain, ×200). B. PASM stain shows the lesion of capillary loops necrosis (arrow).

Table 2. Renal pathological characteristics

	MN with crescents group	Control group	*P* value
Light microscope			
Total no. of glomeruli	27.5 (8-55)	26 (10-55)	0.974
Glomerular sclerosis, %	8.9 (0.0-65.6)	0.0 (0.0-40.0)	0.000
Segmental sclerosis, n (%)	28 (48.3)	16 (16.0)	0.000
Capillary loops necrosis, n (%)	6 (10.3)	0 (0.0)	0.002
Crescents, %	4.6 (1.8-35.3)	0	
Cellular crescents, %	2.4 (0.0-35.3)	0	
Fibrocellular crescents, %	0.0 (0.0-9.1)	0	
Fibrous crescents, %	0.0 (0.0-9.4)	0	
IFTA, n (%)	51 (87.9)	54 (54.0)	0.000
Afferent arterial, n (%)	52 (89.7)	65 (65.0)	0.001
Immunofluorescence			
IgA, n (%)	19 (32.8)	21 (21.0)	0.358
IgM, n (%)	10 (17.2)	19 (19.0)	0.834
C3, n (%)	57 (98.3)	90 (90.0)	0.056
C4, n (%)	3 (5.2)	20 (20.0)	0.010
C1q, n (%)	30 (51.7)	47 (47.0)	0.622
Fibrin, n (%)	7 (12.1)	1 (1.0)	0.004
IgG1, n (%)	40/43 (93.0)	51/54 (94.4)	1.000
IgG2, n (%)	35/43 (81.4)	41/54 (75.9)	0.622
IgG3, n (%)	33/43 (76.7)	32/54 (59.3)	0.084
IgG4, n (%)	41/43 (95.3)	49/54 (90.7)	0.458

P=1.000). The 58 patients with crescentic MN had worse renal function than the 100 patients in the control group: their median levels of serum creatinine and eGFR at renal biopsy were 0.9 vs 0.8 mg/dl and 91.5 vs 107.2 ml/min/1.73 m^2, respectively. Their average serum albumin level was 31.4±6.2 g/l, and the mean protein excretion was 4.3 g/24 h. Thirty-three of these 58 patients (56.9%) had nephropathic proteinuria. There was no difference in urinary sediment between the two groups (**Table 1**).

Renal pathological characteristics

Sampling for light microscopy included a mean of 27.5 glomeruli (range 8 to 55 glomeruli). The proportion of glomerular sclerosis (8.9% vs 0.0%, P=0.000) was higher than that in the control group. The lesions of segmental glomerulosclerosis (48.3% vs 16.0%, P=0.000) and capillary loops necrosis (10.3% vs 0.0%, P=0.002) were more frequent than those in the control group. On average, glomeruli showed 4.6% (range, 1.8%-35.3%) crescents. The cellular, fibrocellular, and fibrous crescents involved a mean of 2.4%, 0.0%, and 0.0% of glomeruli, respectively. The rates of IFTA (87.9% vs 54.0%, P=0.000) and afferent arterial lesions (89.7% vs 65.0%, P=0.001) were higher than those in the control group (**Figure 1**).

Immunofluorescence revealed glomerular capillary wall positivity in all 58 patients for IgG and all but one for C3. The deposition rates of IgA,

Table 3. Circulating PLA_2R autoantibodies and PLA_2R glomerular deposits

	MN with crescents group	Control group	*P* value
Circulating PLA_2R autoantibodies, n (%)	32/50 (64.0)	54/95 (56.8)	0.478
PLA_2R glomerular deposits, n (%)	6/6 (100.0%)	18/18 (100.0%)	

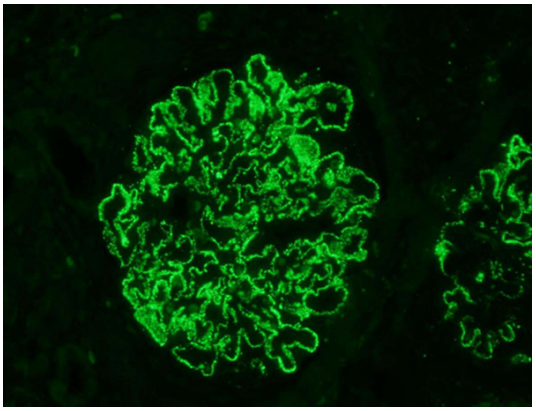

Figure 2. PLA_2R glomerular deposits.

IgM and C1q were 32.8%, 17.2%, and 51.7%, respectively. Weaker staining for C4 (5.2% vs 20.0%, P=0.010) and stronger staining for fibrin (12.1% vs 1.0%, P=0.004) were detected. Immunostaining for IgG subclasses was performed on biopsy specimens from 43 patients; polyclonal glomerular deposits of IgG were observed in all of these specimens (**Table 2**).

PLA_2R

Circulating PLA_2R autoantibodies were detected in 32 of 50 (64.0%) patients. There was no difference in the rate of positive anti-PLA_2R between the two groups (64.0% vs 56.8%, P=0.478). Six biopsies in the MN with crescents group and eighteen biopsies in the control group performed immunostaining for PLA_2R and showed positive granular GBM staining for PLA_2R (**Table 3**; **Figure 2**).

Clinical follow-up and treatment

Clinical follow-up was available for 55 patients in the MN with crescents group and 98 patients in the control group, with a mean follow-up period of 23.0 and 27.0 months, respectively. These patients were treated with renin-angiotensin system blockers, glucocorticoid alone or in combination with cyclophosphamide, tripterygium wilfordii polycoride tablets or calcineurin inhibitors. One patient progressed to end-stage renal disease. The 50 patients had worse renal function than the 98 patients in the control group: their median levels of serum creatinine and eGFR at renal biopsy and the last follow-up were 0.9 vs 0.7 mg/dl, 1.0 vs 0.8 mg/dl, 92.2 vs 107.3 ml/min/1.73 m^2, and 86.3 vs 101.4 ml/min/1.73 m^2, respectively, compared with the control group. There were no differences in the average levels of serum albumin and the mean protein excretion between the two groups. The serum albumin and protein excretion of the 55 patients improved after treatment (**Table 4**).

Discussion

Morphologically, MN is characterized by subepithelial immune complex deposits without the proliferation of glomerular inherent cells and local inflammation. In the setting of membranous glomerulonephritis, endocapillary proliferation, necrosis, and crescent formation are rarely encountered. When present, these changes suggest secondary MN or MN superimposed on anti-GBM nephritis [1-4], systemic vasculitis [5, 6], or IgA nephropathy [7]. Even more rarely, crescents may be encountered in MN in patients lacking evidence of these diseases [8]. Whether these patients present with differing clinical manifestations and outcomes compared with typical IMN patients remains unclear.

The 58 patients had no evidence of secondary MN, and most had positive evidence of anti-PLA_2R. Can these patients be classified as having IMN? As the most common secondary MN, SLE may present with nephritis as the sole manifestation of disease antedating its clinical and immunological markers. Thus, findings of MN with crescents suggest the possibility of SLE. In the series of 10 patients with biopsy-proven MN studied by Adu et al. [9], 4 patients developed extrarenal features suggestive of SLE over a period of one to 14 years. Chen et al. [10] reported 4 such patients. Although they do not demonstrate extrarenal clinical or serological features, patients with crescentic MN (especially those with anti-PLA_2R-negative or ANA-positive serology) should be maintained under surveillance for the appearance of symptoms

Table 4. Clinical follow-up data

	MN with crescents group	Control group	*P* value
Duration, months	23.0 (1.0-70.0)	27.0 (1.0-119.0)	0.359
Serum creatinine at renal biopsy, mg/dl	0.9 (0.4-6.0)	0.7 (0.4-1.8)	0.000
Serum creatinine at the last follow-up, mg/dl	1.0 (0.5-13.4)	0.8 (0.4-7.5)	0.012
eGFR at renal biopsy, ml/min/1.73 m^2	92.2 (7.2-131.6)	107.3 (44.2-159.3)	0.000
eGFR at the last follow-up, ml/min/1.73 m^2	86.3 (4.5-130.2)	101.4 (7.2-151.2)	0.004
Serum albumin at renal biopsy, g/l	31.4±6.3	30.1±6.4	0.222
Serum albumin at the last follow-up, g/l	39.3±8.2	38.2±9.2	0.424
Urinary protein at renal biopsy, g/24 h	4.4 (0.7-22.3)	2.8 (0.5-15.0)	0.350
Urinary protein at the last follow-up, g/24 h	0.9 (0.1-14.2)	0.8 (0.1-14.7)	0.655

and autoantibodies suggestive of autoimmune disease.

The pattern of MN with crescents is occasionally associated with anti-GBM nephritis, systemic vasculitis or IgA nephropathy. In the series, there were 3 cases of MN superimposed on anti-GBM nephritis, 7 cases of ANCA-positive vasculitis, and 2 cases of ANCA-negative vasculitis. They can be diagnosed simultaneously or successively. Rodriguez et al. [8] reported one patient who developed a positive anti-MPO titer 8 months after the kidney biopsy. Multiple cases of MN followed by anti-GBM nephritis have been reported [2-4]. Therefore, repeat testing for ANCA and anti-GBM and surveillance for extrarenal presentation in these patients may be warranted. Additionally, 5 patients had overlapping IgA nephropathy and MN. The renal biopsy specimens revealed diffuse mesangial proliferation and mesangial-intense immunostaining for IgA nephropathy. The crescents may be ascribed to IgA nephropathy. Cases of MN superimposed on IgA nephropathy are rare [7, 11]. Wang et al. [7] reported 11 cases with overlapping IgA nephropathy and MN, and occasional glomeruli with crescent formation were identified in 2. Therefore, IgA nephropathy should be taken into account when IgA deposits in the mesangium are observed in crescentic MN.

Additionally, the crescents in MN may be attributed to monoclonal gammopathy. Various lesions have been found in patients with monoclonal gammopathy, including membranoproliferative glomerulonephritis, endocapillary proliferative glomerulonephritis, focal segmental glomerulosclerosis, interstitial nephritis, immunotactoid glomerulopathy, and MN [12-14]. Some cases can exhibit crescents [15], which may be due to a diverse spectrum of renal injury related to the renal parenchymal deposition of monoclonal Igs or their subunits. However, most patients in the present series showed polyclonal immune complex deposits; these cases therefore do not represent monoclonal gammopathy.

The detection of circulating PLA_2R autoantibodies and immunostaining for PLA_2R have been proposed as methods to discriminate primary from secondary MN because circulating antibodies directed against PLA_2R are present in 70% of patients with primary MN [16]. In the crescentic MN study by Rodriguez et al. [8], 38% of biopsy specimens showed positive staining for PLA_2R. Rodriguez et al. [8] asserted that at least a subset of these patients has primary MN and that primary MN can also show crescents, perhaps due to severe glomerular damage related to immune complex deposition. In the present study, circulating PLA_2R autoantibodies were detected in 32 of 50 (64.0%) patients, and positive staining for PLA_2R were observed in six biopsies of the MN with crescents group. These patients can thus be diagnosed as having IMN. Additionally, Tomas et al. [17] identified THSD7A as the second autoantigen involved in adult IMN. For anti-PLA_2R-negative MN, serologic testing for anti-THSD7A autoantibodies and histologic staining for THSD7A can further help in distinguishing between idiopathic and secondary MN.

In summary, in MN with crescents, SLE, anti-GBM nephritis, systemic vasculitis, and IgA nephropathy should first be considered, and

the extrarenal presentations and autoantibodies should be closely monitored during follow-up. At least a portion of patients with crescentic MN can be determined to have IMN. This may represent a distinct clinicopathologic entity that usually presents with hypertension and decreased eGFR clinically, severe capillary loops necrosis and IFTA histologically, with a favorable prognosis in short-term follow-up.

As a retrospective single-center study, this study had some limitations, such as the small number of cases, the incomplete clinicopathological data, and a short follow-up. Long-term observations of the series and further study of the pathogenesis are required to further our understanding and evaluate the long-term prognosis of MN with crescents.

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Disclosure of conflict of interest

None.

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