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ORIGINAL ARTICLE

Clinicopathological and long-term prognostic features of membranous nephropathy with crescents: a Japanese single-center experience

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Abstract

Background Three recent studies from the United States and China reported the clinicopathological features and short-term prognosis in patients with membranous nephropathy (MN) and crescents in the absence of secondary MN, anti-glomerular basement membrane (GBM) antibodies, and anti-neutrophil cytoplasmic antibodies (ANCA).

Methods We compared clinicopathological and prognostic features in 16 MN patients with crescents (crescent group) and 38 MN patients without crescents (control group), in the absence of secondary MN, anti-GBM antibodies, and ANCA. Median follow-up periods in the crescent and control groups were 79 and 50 months, respectively.

Results Decreased estimated glomerular filtration rates (<50 mL/min/1.73 m^2), glomerulosclerosis, and moderate-to-severe interstitial fibrosis were more frequently observed in the crescent group than in the control group ($P = 0.043$, $P = 0.004$, and $P = 0.035$, respectively). Positive staining rates for glomerular IgG2 and IgG4 were significantly different between the 2 groups ($P = 0.032$, $P = 0.006$, respectively). Doubling of serum creatinine during follow-up was more frequently observed in the crescent group than in the control group ($P = 0.002$), although approximately two-thirds of patients in the crescent group were treated with immunosuppressive therapy. Crescent formation and interstitial fibrosis were risks for doubling of serum creatinine [hazard ratio (HR) = 10.506, $P = 0.012$; HR = 1.140, $P = 0.009$, respectively].

Conclusions This is the first Japanese study demonstrating significant differences in clinicopathological and prognostic features between the 2 groups. Most patients in the crescent group may develop a long-term decline in renal function despite immunosuppressive therapy.

Keywords Clinicopathological study · Crescent formation · IgG-subclass · Membranous nephropathy · Prognosis

Introduction

Membranous nephropathy (MN) is characterized by glomerular basement membrane (GBM) thickening and subepithelial immune deposits [1]. MN is classified into primary or idiopathic (I-MN) and secondary (S-MN). Most cases are primary, but MN may be secondary to lupus nephritis, infections, cancers, and drugs [1]. It is known that M-type phospholipase A2 receptor (PLA2R) is the major podocyte target antigen in I-MN patients [2]. In I-MN cases, PLA2R-associated cases are considered to be ~80% [1]. The variability in sensitivity may be related to ethnicity (e.g., Japanese patients with I-MN have a lower rate of anti-PLA2R positivity [3, 4]).

Human IgG is divided into 4 subclasses (IgG1, IgG2, IgG3, and IgG4), and each subclass differs in complement-activating ability [5]. Several studies, including our previous studies, have shown different distribution patterns of glomerular IgG-subclass depositions between I-MN and S-MN associated with systemic lupus erythematosus [6], mixed connective tissue disease [7], anti-rheumatic drug

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bucillamine [8], and malignancy [9]. These findings suggest that I-MN and S-MN may result from different immunological mechanisms.

Crescent formation appears to represent a nonspecific response to severe injury to the glomerular capillary wall, and is usually due to anti-GBM antibody diseases, immune complex-related diseases, or anti-neutrophil cytoplasmic antibodies (ANCA)-associated diseases [10]. Early therapy based on a combination of steroids and cytotoxic drugs is important to improve renal outcomes of crescentic glomerulonephritis [10].

In MN patients without evidence of S-MN, crescent formation is a rare complication unless they have a superimposed glomerulonephritis attributable to anti-GBM antibodies [11] and ANCA [12, 13]. In addition, there are few reported MN cases with crescent formation, in the absence of S-MN, anti-GBM antibodies, and ANCA [14–18]. Three recent studies from the United States [19] and China [20, 21] analyzed the clinicopathological features at presentation and short-term renal outcomes of a group of patients with this MN variant. Rodriguez et al. [19] and Wang et al. [20] suggested an unfavorable prognosis, while Qian et al. [21] suggested a favorable prognosis. The effects of crescent formation on renal outcomes are still unclear. Furthermore, there is only 1 study focusing on the distribution patterns of glomerular IgG-subclass depositions in these patients [21].

In the present Japanese cohort study, we examined clinicopathological features, including the distribution patterns of glomerular IgG-subclass depositions and long-term renal outcomes, of 16 MN patients with crescents and 38 MN patients without crescents in the absence of S-MN, anti-GBM antibodies, and ANCA.

Patients and methods

Patients

This study was based on the renal histological records (from June 1991 to August 2014) of 5919 patients studied at Akita University Hospital and its affiliated hospitals. Among these patients, 520 patients (8.8%) were diagnosed with MN.

Clinical and laboratory data were collected at the time of renal biopsy. To determine the outcome in each patient after biopsy, follow-up laboratory data and treatment information were collected via a questionnaire. Nephrotic syndrome was defined as urinary protein $\geq$3.5 g/day or g/g creatinine (Cr), hypoalbuminemia (serum albumin $\leq$3.0 g/dL), and edema. Estimated glomerular filtration rate (eGFR) was calculated using the formula for Japanese patients [22]. Decreased eGFR was defined as <60 mL/min/1.73 m^2, as in the previous studies [19–21], or as <50 mL/min/1.73 m^2. In all patients in the crescent group (described below), circulating anti-GBM antibodies, myeloperoxidase-ANCA, and proteinase 3-ANCA were evaluated by enzyme-linked immunosorbent assays in the laboratory of Special Reference Laboratories, Tokyo, Japan. Circulating anti-PLA2R antibodies were detected by an indirect immunofluorescence assay system (EUROIMMUN AG, Lübeck, Germany) [23].

Figure 1 shows the cohort selection in this study. I-MN was defined as MN in the absence of known clinical and immunological factors causing S-MN [1]. Among 520 biopsy-proven MN patients, 325 patients were considered to have I-MN. Patients with clinical evidence of known S-MN [1] were excluded. Patients with positive glomerular staining for C1q were also excluded, considering the

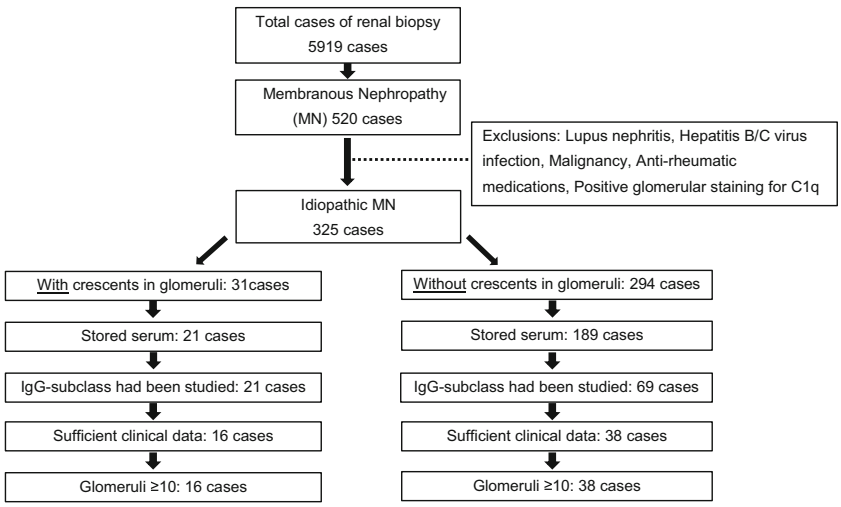

Fig. 1 Cohort selection based on the inclusion and exclusion criteria used in this study

Table 1 Clinical features of patients in the crescent group

Patient no.	At the time of renal biopsy											Treatment	Follow-up				
	Age	Sex	Clinical symptoms	HT	UP (g/day or g/gCr)	Hem	S-Alb (g/dL)	S-Cr (mg/dL)	eGFR (mL/min/1.73 m^2)	CRP (mg/dL)	Anti-PLA2R Ab		Time (months)	UP (g/day or g/gCr)	S-Alb (g/dL)	S-Cr (mg/dL)	eGFR (mL/min/1.73 m^2)
1	49	M	P	+	3.0	–	3.6	0.6	111.0	0.0	–	PSL, CYC, RAS	223	1.9	2.9	1.0	60.0
2	45	M	P	–	10.0	+	3.4	1.3	48.8	0.0	+	PSL, CYC	290	4.2	3.6	2.6	20.2
3	39	M	P	+	3.3	–	2.9	0.9	76.1	0.1	–	PSL	280	2.8	3.4	1.8	31.2
4	63	M	P	+	2.6	–	4.5	1.3	44.3	0.1	–	PSL, CYC	167	1.1	3.6	2.4	21.4
5	55	M	NS	+	13.4	+	2.2	0.7	90.7	0.1	–	PSL, CYC, RAS	92	0.1	4.2	0.7	90.1
6	42	F	NS	–	10.2	+	2.4	0.5	104.7	0.0	+	PSL, CYC, RAS	92	0.1	4.1	0.5	91.5
7	52	M	H/P	+	2.5	+	3.6	0.9	70.0	0.1	–	PSL, CYC, RAS	92	1.2	4.1	1.3	45.7
8	66	M	NS	+	17.0	+	2.1	1.8	30.6	0.1	–	PSL, RAS	81	1.0	4.2	1.2	47.7
9	75	F	NS	+	4.7	+	2.8	0.7	61.3	0.2	+	PSL, CYC, RAS	77	0.3	4.3	1.5	25.3
10	47	M	NS	+	6.7	+	2.0	1.5	41.2	0.4	+	PSL, MZR, RAS	71	0.1	4.3	3.2	17.3
11	73	M	P	+	1.4	–	4.2	0.7	83.7	0.1	–	RAS	70	0.4	4.3	1.9	27.9
12	62	M	NS	+	7.3	+	2.5	1.3	44.5	0.8	+	PSL, CyA, RAS	72	2.5	4.5	3.2	16.1
13	50	M	NS	+	9.9	–	1.6	0.9	70.8	0.4	+	PSL, CyA, RAS	56	0.2	3.6	1.3	47.7
14	58	M	P	–	0.5	–	4.3	0.8	77.2	0.0	–	RAS	46	0.1	4.1	0.7	82.5
15	80	M	NS	–	3.6	+	1.8	1.1	49.7	2.4	+	PSL, CYC	43	0.5	3.8	1.0	57.5
16	67	M	NS	+	10.7	+	2.8	1.1	50.8	0.0	–	PSL, RAS	24	0.6	4.0	1.3	39.8

Ab antibody, *Cr* creatinine, *CRP* C-reactive protein, *CyA* cyclosporine A, *CYC* cyclophosphamide, *eGFR* estimated glomerular filtration rate, *Hem* hematuria, *H/P* hematuria and proteinuria, *HT* hypertension, *MZR* mizoribine, *NS* nephrotic syndrome, *P* proteinuria, *PLA2R* phospholipase A2 receptor, *PSL* prednisolone, *RAS* renin–angiotensin system blockade, *S-Alb* serum albumin, *S-Cr* serum creatinine, *UP* urinary protein

suggestion that I-MN may be distinguished from S-MN by its lack of C1q [1]. Among 325 I-MN patients, 31 patients (9.5%) had crescent formation. Those meeting any of the following criteria were further excluded from this study: (1) there was no remaining serum for immunological analyses for anti-GBM antibodies, ANCA, and anti-PLA2R antibodies; (2) distribution of glomerular IgG-subclass depositions had not been studied; (3) there was not sufficient clinical data such as levels of urinary protein excretion, serum levels of albumin and Cr at the time of biopsy, or detailed information on treatment outcomes; or (4) insufficient number of glomeruli (<10) in biopsy specimens for light microscopy. Based on these criteria, 16 patients with crescents (crescent group) and 38 patients without crescents (control group) were enrolled in this study. All patients in the crescent group had negative anti-GBM antibodies and negative ANCA. The difference of the selection rate of patients between the crescent and control groups largely depended on the number of excluded patients in the control group, in whom IgG-subclass analysis had not been performed.

Pathological studies

The renal biopsy specimens were processed using standard techniques for light, immunofluorescence, and electron microscopy. The interstitial fibrosis area ratio on Masson trichrome-stained specimens was quantitatively evaluated using WinRoof version 5.5.0 software (Mitani Co., Fukui, Japan), and categorized as mild (<25%), moderate (25–50%), or severe (>50%), as previously described [24]. Tubular atrophy and small round cell infiltration were semi-quantitatively graded as mild (<25%), moderate (25–50%), or severe (>50%). Determination of IgG-subclass depositions was performed on cryostat sections, as described previously [25].

Treatments and therapeutic responses

Most patients, except for patients with mild proteinuria and normal renal function, were treated with steroids. Combination therapies with immunosuppressive agents (cyclophosphamide, cyclosporine A, or mizoribine) were selected for unsuccessfully controlled cases with steroids alone. Patients with hypertension were treated mainly with renin–angiotensin system (RAS) blockers. Therapeutic responses in these patients were evaluated according to the 2011 KDIGO Guidelines [26]. For evaluation of renal outcomes, the primary endpoint was end-stage renal disease (ESRD) requiring dialysis; the second point was doubling of serum Cr.

Statistical analysis

The difference in both groups was analyzed by Student's *t* test, Welch's *t* test, Mann–Whitney's *U* test, or Pearson's Chi-square test. Renal outcome curves were constructed using the Kaplan–Meier method. The log-rank test was used to evaluate the differences between the 2 groups. Univariate and multivariate logistic regression analyses were used to determine which clinicopathological data were associated with treatment response. Univariate and multivariate Cox regression analyses were performed in order to determine factors affecting renal outcomes. All analyses were performed with SPSS version 11.0 software for Windows (Chicago, IL, USA). P values < 0.05 were considered significant in all analyses.

Results

Clinicopathological features in the crescent group

Clinicopathological features of 16 patients in the crescent group at the time of biopsy are summarized in Tables 1, 2, 3, and 4.

The mean age was 57.7 years. Fourteen patients were male and 2 patients were female. Nine (56%) patients had nephrotic syndrome. Twelve (75%) patients were complicated by hypertension. All patients presented proteinuria (median, 5.7 g/day or g/gCr), and 10 (63%) patients had microscopic hematuria. The mean serum albumin was 2.9 g/dL, and the mean eGFR was 66.0 mL/min/1.73 m^2. The mean serum C-reactive protein, C3, and C4 were 0.1, 107.6, and 33.5 mg/dL, respectively. Serum anti-PLA2R antibodies were positive in 7 (44%) patients.

The average number of glomeruli was 27 per biopsy, with the average of 19% global sclerosis. The average rate of glomerular crescent formation was 5%. Among 16 patients, 9 patients (56%) had moderate-to-severe interstitial fibrosis, 5 (31%) patients had moderate tubular atrophy, and 9 patients (56%) had moderate-to-severe small round cell infiltration. MN stages were evaluated by electron microscopy in 12 patients. Among them, 3 (25%) patients were diagnosed with MN stage I, 7 (58%) patients with MN stage II, and 2 (17%) patients with MN stage III. Mesangial electron-dense deposits suggestive of S-MN [27] were not observed, as well as in the control group. By immunofluorescence, all biopsy specimens exhibited granular staining for IgG, κ, and λ, along the glomerular capillary loops. Positive granular staining for IgG1, IgG2, IgG3, and IgG4 was observed in 2 (13%) patients, 15 (94%) patients, 3 (19%) patients, and 13 (81%) patients, respectively.

Table 2 Pathological features of patients in the crescent group

Patient no.	Total no. of glomeruli	Sclerotic glomeruli (%)	Crescentic glomeruli (%)	Interstitial fibrosis (%)	Tubular atrophy	Interstitial SRC infiltration	MN stage	Immunofluorescence study						IgG subclass			
								IgG	IgA	IgM	κ	λ	C3	G1	G2	G3	G4
1	18	16.7	5.6	14.4	Mild	Mild	II	3	0	0	2	1	1	0	1	0	1
2	28	3.6	10.7	17.8	Mild	Mild	II	3	Trace	Trace	2	2	1	0	1	1	2
3	31	22.6	12.9	21.5	Mild	Moderate	NA	3	0	1	2	1	1	0	1	0	0
4	26	42.3	15.4	25.4	Moderate	Moderate	NA	3	3	0	3	3	0	0	1	0	0
5	24	8.3	4.2	17.8	Mild	Mild	I	3	Trace	0	3	2	1	0	3	0	3
6	30	6.7	3.3	15.5	Mild	Mild	III	2	0	0	1	1	1	0	1	Trace	3
7	16	31.3	6.3	12.2	Mild	Moderate	III	2	1	Trace	2	2	1	0	1	0	0
8	30	43.3	3.3	33.2	Moderate	Severe	II	2	0	0	2	1	2	0	0	0	2
9	10	20.0	10.0	30.0	Mild	Mild	NA	3	0	1	3	3	3	0	1	0	2
10	20	5.0	5.0	38.7	Mild	Moderate	I	3	0	0	3	3	2	1	1	1	2
11	27	40.7	3.7	44.4	Moderate	Moderate	II	3	0	0	3	3	2	1	1	1	2
12	10	40.0	10.0	52.1	Moderate	Severe	I	3	0	0	3	3	0	0	2	0	3
13	16	18.8	6.3	42.4	Mild	Moderate	NA	3	3	0	3	3	1	0	2	0	3
14	49	2.0	2.0	28.3	Mild	Mild	II	3	3	Trace	2	2	0	0	1	0	2
15	44	15.9	2.3	19.7	Mild	Mild	II	3	0	0	3	3	3	0	1	0	2
16	29	51.7	3.4	34.9	Moderate	Moderate	II	1	Trace	1	1	1	0	0	1	0	2

Tubular atrophy and interstitial SRC infiltration were graded semi-quantitatively as mild (<25%), moderate (25–50%), and severe (>50%). Glomerular staining intensity was graded as negative (Score 0), weakly positive (trace), 1+ (Score 1), 2+ (Score 2), and 3+ (Score 3)

MN membranous nephropathy, *NA* no assessment, *SRC* small round cell

Table 3 Clinical and laboratory features of patients in the crescent and control groups

	Crescent (no. = 16)	Control (no. = 38)	*P* value
Age	57.7 (±12.3)	63.5 (±11.3)	0.098[a]
Sex (M/F)	14/2	22/16	0.035[b]
Nephrotic syndrome, no. (%)	9 (56.3)	22 (57.9)	0.911[b]
Hypertension, no. (%)	12 (75.0)	29 (76.3)	0.918[b]
Proteinuria (g/d or g/gCr)	5.7 (0.5–17.0)	4.3 (0.5–20.0)	0.240[c]
Hematuria, no. (%)	10 (62.5)	17 (44.7)	0.233[b]
Serum albumin, g/dL	2.9 (±0.9)	2.7 (±0.9)	0.394[a]
eGFR, mL/min/1.73 m^2	66.0 (±23.6)	72.3 (±24.7)	0.384[a]
Declined eGFR (<60 mL/min/1.73 m^2), no. (%)	7 (43.8)	9 (23.7)	0.140[b]
Declined eGFR (<50 mL/min/1.73 m^2), no. (%)	6 (37.5)	5 (13.2)	0.043[b]
Hemoglobin (g/dL)	13.6 (±1.9)	13.0 (±1.8)	0.284[d]
Serum CRP (mg/dL)	0.1 (0–2.4)	0.0 (0–0.4)	0.070[c]
Serum C3 (mg/dL)	107.6 (±7.6)	118.3 (±3.5)	0.122[d]
Serum C4 (mg/dL)	33.5 (±2.8)	32.1 (±1.6)	0.827[d]
Serum anti-PLA2R Ab positive, no. (%)	7 (43.7)	10 (26.3)	0.208[b]

Ab antibody, *Cr* creatinine, *CRP* C-reactive protein, *eGFR* estimated glomerular filtration rate, *F* female, *M* male, *PLA2R* phospholipase A2 receptor

[a] Welch's *t* test

[b] Pearson's Chi-square

[c] Mann–Whitney's *U* test

[d] Student's *t* test

Comparison of clinicopathological features between the crescent and control groups

Table 3 summarizes clinical features at the time of renal biopsy in the crescent and control groups. The proportion of male patients in the crescent group was significantly higher than that in the control group (88 vs. 58%, $P = 0.035$). Decreased eGFR (<50 mL/min/1.73 m^2) was more frequently observed in the crescent group than in the control group (38 vs. 13%, $P = 0.043$). There were no other significant differences between the 2 groups, including positive rates of circulating anti-PLA2R antibodies.

Table 4 summarizes the pathological features in the 2 groups. Although there was no difference in the average number of glomeruli in renal specimens between the 2 groups, global glomerulosclerosis was more frequently observed in the crescent group than in the control group (19 vs. 7%, $P = 0.004$). There was no significant difference in MN stages between the 2 groups. Moderate-to-severe interstitial fibrosis, tubular atrophy, and small round cell infiltration were more frequently observed in the crescent group than in the control group (56 vs. 26%, $P = 0.035$; 31 vs. 3%, $P = 0.002$; and 56 vs. 8%, $P < 0.001$, respectively). There were no significant differences in staining for glomerular IgG, IgA, IgM, κ, λ, or C3 between the 2 groups. On the contrary, the rate of positive staining for IgG2 was significantly higher in the crescent group than in the control group (94 vs. 66%, $P = 0.032$), and the rate of positive staining for IgG4 was significantly lower in the crescent group than in the control group (81 vs. 100%, $P = 0.006$).

Treatments and renal outcomes in the crescent and control groups

Table 5 summarizes treatment outcomes in the crescent and control groups during the follow-up period. There was no significant difference in the median follow-up period between the 2 groups. Patients in the crescent group were more aggressively treated with steroids plus immunosuppressive agents than in the control group (69 vs. 32%, $P = 0.012$). There was no significant difference in the use of RAS blockade between the 2 groups. There were no significant differences in complete remission (CR) rates or partial remission (PR) rates between the 2 groups. There were no significant differences in relapsing rates after achieving remission between the 2 groups. During the observation period, there was no patient who progressed to ESRD requiring dialysis in the 2 groups. However, the frequency of doubling of serum Cr in the crescent group was significantly higher than that in the crescent group (38 vs. 5%, $P = 0.002$). Kaplan–Meier analysis also demonstrated that long-term renal outcome defined as doubling of serum Cr was worse in the crescent group than in the control group ($P = 0.019$) (Fig. 2). Long-term renal

Table 4 Pathological features of patients in the crescent and control groups

	Crescent (no. = 16)	Control (no. = 38)	*P* value
Total no. of glomeruli	27 (10–49)	27 (10–77)	0.483[a]
Sclerotic glomeruli (%)	19.4 (2.0–51.7)	7.2 (0–83.1)	0.004[a]
Crescent (%)	5.3 (2.0–15.4)	–	<0.001[a]
MN stage			
Stage I, no. (%)	3 (25.0)	6 (18.2)	0.613[b]
Stage II, no. (%)	7 (58.3)	20 (60.6)	0.891[b]
Stage III, no. (%)	2 (16.7)	7 (21.2)	0.736[b]
Stage IV, no. (%)	0	0	–
No assessment	4	5	
Interstitial fibrosis (%)			0.035[b]
Mild (<25%)	7 (43.8)	28 (73.7)	
Moderate to severe (≥25%)	9 (56.2)	10 (26.3)	
Tubular atrophy (%)			0.002[b]
Mild (<25%)	11 (68.8)	37 (97.4)	
Moderate (25–50%)	5 (31.2)	1 (2.6)	
Interstitial SRC infiltration (%)			<0.001[b]
Mild (< 25%)	7 (43.8)	35 (92.1)	
Moderate to severe (≥25%)	9 (56.2)	3 (7.9)	
Immunofluorescence study			
IgG positive, no. (%)	16 (100)	38 (100)	–
IgA positive, no. (%)	4 (25)	7 (18.4)	0.584[b]
IgM positive, no. (%)	3 (18.8)	9 (23.7)	0.690[b]
κ positive, no. (%)	16 (100)	38 (100)	–
λ positive, no. (%)	16 (100)	38 (100)	–
C3 positive, no. (%)	12 (75)	27 (71.1)	0.767[b]
IgG subclass			
G1 positive, no. (%)	2 (12.5)	6 (15.8)	0.756[b]
G2 positive, no. (%)	15 (93.8)	25 (65.8)	0.032[b]
G3 positive, no. (%)	3 (18.8)	8 (21.1)	0.848[b]
G4 positive, no. (%)	13 (81.3)	38 (100)	0.006[b]

SRC small round cell

[a] Mann–Whitney's *U* test

[b] Pearson's Chi-square

Table 5 Therapeutic responses and renal outcomes in patients in the crescent and control groups

	Crescent (no. = 16)	Control (no. = 38)	*P* value
Steroids, no. (%)	14 (87.5)	31 (81.6)	0.594[a]
Steroids + immunosuppressive agents, no. (%)	11 (68.8)	12 (31.6)	0.012[a]
Renin–angiotensin system blockade, no. (%)	12 (75.0)	26 (68.4)	0.629[a]
Complete remission, no. (%)	10 (62.5)	24 (63.2)	0.964[a]
Partial remission, no. (%)	6 (37.5)	10 (26.3)	0.411[a]
No remission, no. (%)	0 (0)	4 (10.5)	0.177[a]
Term for remission (mo)	14 (1–91)	9 (1–84)	0.126[b]
Relapse, no. (%)	5 (31.3)	7 (18.4)	0.300[a]
Doubling of serum creatinine, no. (%)	6 (37.5)	2 (5.3)	0.002[a]
Follow-up term (mo)	79 (24–290)	50 (17–258)	0.062[b]

[a] Pearson's Chi-square

[b] Mann–Whitney's *U* test

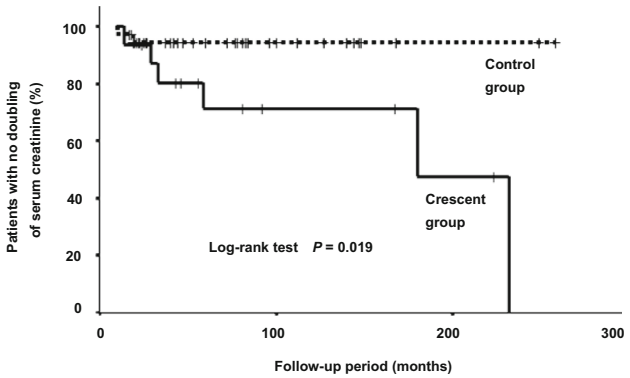

Fig. 2 Kaplan–Meier analysis for doubling of serum creatinine in the crescent group and the control group during follow-up. Patients in the crescent group had worse renal outcomes ($P = 0.019$)

outcomes were further compared between the 2 groups by comparing patients with the similar degree of interstitial fibrosis. Kaplan–Meier analysis showed that there were no significant differences in renal outcomes between the 2 groups of patients with mild interstitial fibrosis ($P = 0.477$) and patients with moderate-to-severe interstitial fibrosis ($P = 0.200$).

Table 6 shows the results of logistic regression analyses to explain significant predictors of the risk for difficulty in achieving CR after treatment in the crescent and control groups. Univariate analysis showed that a higher proportion of global glomerulosclerosis was a predictor of difficulty in achieving CR. This result was confirmed by multivariate analysis model 1 [odds ratio (OR): 1.073; 95% confidence interval (CI): 1.010–1.140; $P = 0.023$] and model 2 (OR: 1.087; 95% CI: 1.014–1.166; $P = 0.019$).

Table 7 shows the results of Cox regression analyses to explain significant predictors of the risk for doubling of serum Cr after treatment in the crescent and control groups. Univariate analysis revealed that higher proportions of crescent formation and interstitial fibrosis area were predictors of the risk for doubling of serum Cr. These results were confirmed by multivariate analysis model 1 and model 2. In both models, a higher proportion of crescent formation was a predictor of the risk for doubling serum Cr [hazard ratio (HR): 10.506; 95% CI: 1.678–65.773; $P = 0.012$]. A higher proportion of interstitial fibrosis was also a predictor of the risk for doubling of serum Cr (HR: 1.140; 95% CI: 1.034–1.258; $P = 0.009$).

Table 6 Predictors of the risk for non-complete remission after treatment in the crescent and control groups

	Univariate models			Multivariate model 1[a]			Multivariate model 2[b]		
	OR	95% CI	*P* value	OR	95% CI	*P* value	OR	95% CI	*P* value
Age	1.021	0.973–1.071	0.408						
Sex (male)	1.126	0.351–3.614	0.842						
Hypertension	0.923	0.923–3.388	0.903						
Proteinuria (increased by 1 g/d)	1.068	0.941–1.211	0.309						
Hematuria	1.000	0.331–3.018	1.000						
Serum albumin (increased by 1 g/dL)	0.822	0.435–1.552	0.545						
eGFR (increased by 1 mL/min/1.73 m^2)	0.990	0.965–1.015	0.424						
Serum anti-PLA2R Ab positive	1.636	0.477–5.613	0.434						
Crescent formation	0.972	0.291–3.253	0.964						
Percentage of global sclerotic glomeruli (increased by 1%)	1.054	1.009–1.099	0.017	1.073	1.010–1.140	0.023	1.087	1.014–1.166	0.019
Percentage of interstitial fibrosis (increased by 1%)	1.058	0.996–1.124	0.069						
Immunosuppressive therapy (PSL or any other immunosuppressive agents)	2.333	0.435–12.530	0.323						
RAS blockade therapy	1.636	0.477–5.613	0.434						

Model 1: good suitability (X^2: 5.235, $P = 0.73$), Model 2: good suitability (X^2: 4.393, $P = 0.82$)

Ab antibody, *CI* confidence interval, *eGFR* estimate glomerular filtration rate, *OR* Odds ratio, *PLA2R* phospholipase A2 receptor, *PSL* prednisolone, *RAS* renin–angiotensin system. Univariate and multivariate logistic regression analyses explain significant predictors of the risk for non-complete remission. The suitability of the two models was good by Hosmer–Lemeshow test

[a] Multivariate with age, sex, hypertension, proteinuria, hematuria, serum albumin, eGFR, Anti-PLA2R Ab, Crescent formation, percentage of global sclerotic glomeruli, Percentage of interstitial fibrosis

[b] Multivariate with age, sex, hypertension, proteinuria, hematuria, serum albumin, eGFR, anti-PLA2R Ab, crescent formation, Percentage of global sclerotic glomeruli, percentage of interstitial fibrosis, immunosuppressive therapy, RAS blockade therapy

Table 7 Predictors of the risk for doubling of serum creatinine after treatment in the crescent and control groups

	Univariate models			Multivariate model 1[a]			Multivariate model 2[b]		
	HR	95% CI	*P* value	HR	95% CI	*P* value	HR	95% CI	*P* value
Age	1.046	0.971–1.127	0.239						
Sex (male)	0.732	0.174–3.087	0.671	0.110	0.014–0.839	0.033	0.110	0.014–0.839	0.033
Hypertension	2.692	0.328–22.102	0.357						
Proteinuria (increased by 1 g/d)	1.076	0.935–1.239	0.308						
Serum albumin (increased by 1 g/dL)	0.768	0.315–1.875	0.562						
eGFR (increased by 1 mL/min/1.73 m^2)	0.968	0.934–1.239	0.082						
Anti-PLA2R Ab positive	3.624	0.804–16.346	0.094						
Crescent formation	5.654	1.125–28.423	0.035	10.506	1.678–65.773	0.012	10.506	1.678–65.773	0.012
Percentage of global sclerotic glomeruli (increased by 1%)	1.028	0.991–1.066	0.136						
Percentage of interstitial fibrosis (increased by 1%)	1.089	1.027–1.154	0.004	1.140	1.034–1.258	0.009	1.140	1.034–1.258	0.009
Relapse	4.125	0.927–19.155	0.063						
Immunosuppressive therapy (PSL or any other immunosuppressive agents)	0.824	0.095–7.146	0.860						
RAS blockade therapy	2.270	0.435–11.848	0.331						

Univariate and multivariate Cox regression analyses to explain significant predictors of the risk for doubling of serum creatinine

Ab antibody, *CI* confidence interval, *eGFR* estimate glomerular filtration rate, *HR* hazard ratio, *PLA2R* phospholipase A2 receptor, *PSL* prednisolone, *RAS* renin–angiotensin system

[a] Multivariate with age, sex, hypertension, eGFR, proteinuria, serum albumin, anti-PLA2R antibody, crescent formation, percentage of global sclerotic glomeruli, percentage of interstitial fibrosis, relapse

[b] Multivariate with age, sex, hypertension, eGFR, proteinuria, serum albumin, anti-PLA2R antibody, crescent formation, percentage of global sclerotic glomeruli, percentage of interstitial fibrosis, relapse, immunosuppressive therapy, RAS blockade therapy

Discussion

The present study demonstrated for the first time the clinicopathological features and long-term treatment outcomes in Japanese patients with MN and crescents, in the absence of S-MN, anti-GBM antibodies, and ANCA. We compared clinicopathological and prognostic features between 16 MN patients with crescents (crescent group) and 38 MN patients without crescents (control group). We also compared the distribution patterns of glomerular IgG-subclass depositions between the 2 groups.

Table 8 summarizes the clinical features at presentation in previously reported cases and our cases of MN with crescents, in the absence of S-MN, anti-GBM antibodies, and ANCA. There was a male predominance in Rodriguez's cohort [19], Qian's cohort [21], and our cohort. The mean ages of patients were 50.2–57.7 years. All patients presented with proteinuria (4.3–11.5 g/day or g/gCr), and 63–89% of patents had hematuria. The mean serum levels of albumin and Cr were 2.4–3.1 g/dL and 0.7–2.9 mg/dL, respectively. Decreased eGFR (<60 mL/min/1.73 m^2) was observed in 79, 21, and 26% of patients in Rodriguez's cohort [19], Wang's cohort [20], and Qian's cohort [21], respectively. In our cohort, 44% of patients had eGFR of <60 mL/min/1.73 m^2 and 38% of patients had eGFR of <50 mL/min/1.73 m^2. There was a significant difference in decreased eGFR defined as <50 mL/min/1.73 m^2 between the crescent and control groups. In Wang's cohort [20], Qian's cohort [21], and our cohort, there was no significant difference in the rate of positive PLA2R antibodies between the 2 groups. This suggests that PLA2R antibodies may not be related to crescent formation. In our Japanese cohort, positive PLA2R antibodies were detected in 26.3–43.7% of patients in the crescent and control groups, by using the same assay system. It is known that Japanese patients with I-MN have a lower rate of anti-PLA2R positivity [3, 4].

Regarding pathological features, comparative studies between the crescent and control groups were performed in Wang's cohort [20], Qian's cohort [21], and our cohort. Among these cohorts, the rates of crescent formation in the crescent group were similar (4.6–5.3%). In Qian's cohort [21], the rate of positive staining for IgG3 was significantly higher in the crescent group than in the control group. In our

Table 8 Cases of MN with crescents without anti-GBM Ab and ANCA

References	Year	Patient no.	Age	Sex (M:F)	Urinary protein (g/day or g/gCr)	Hematuria (%)	Serum albumin (g/dL)	Serum creatinine (mg/dL)	Decreased eGFR[a] no. (%)	Ccr/eGFR (mL/min or mL/min/1.73 m^2)	Anti-PLA2R Ab positive (%)
Kwan [14]	1991	1	38	M	17.0	ND	2.5	2.0		59	ND
Tse [15]	1997	3	39/41/58	M/M/F	ND/20.6/3.5	Yes/no/yes	1.5/1.3/2.3	19.8/1.6/0.7		ND/86/100	ND
Arrizabalaga [16]	1998	2	83/49	1:1	6.0/3.8	100	2.1/1.3	10/3.2		ND	ND
Unver [17]	2008	1	65	F	4.7	Yes	2.5	6.3		ND	ND
Gadonski [18]	2010	1	18	M	6.4	Yes	1.8	6.4		ND	ND
Rodriguez [19]	2014	19	54.8 ± 23.3	11:8	11.5 (3.3–29.0)	88.9	2.4 ± 0.6	2.9 (0.4–10.0)	15 (78.9)	39.7 (4.0–119.0)	37.5
Wang [20]	2015	28	56.0 ± 11.1	11:17	6.5 ± 4.8	89.3	2.7 ± 0.8	0.7 ± 0.2	6 (21.4)	ND	79.7
Qian [21]	2016	58	50.2 ± 14.3	37:21	4.3 (0.7–22.3)	ND	3.1 ± 0.6	0.9 (0.4–6.0)	15 (25.9)	91.5 (7.2–131.6)	64.0
Present study		16	57.7 ± 12.3	14:2	5.7 (0.5–17.0)	62.5	2.9 ± 0.9	1.0 ± 0.4	7 (43.8)	66.0 ± 23.6	43.7

Ab antibodies, *ANCA* anti-neutrophil cytoplasmic antibodies, *Ccr* creatinine clearance, *Cr* creatinine, *eGFR* estimated glomerular filtration rate, *GBM* glomerular basement membrane, *MN* membranous nephropathy, *ND* no data, *PLA2R* phospholipase A2 receptor

[a] eGFR of <60 mL/min/1.73 m^2

cohort, the rate of positive staining for IgG2 was significantly higher in the crescent group than in the control group, and the rate of positive staining for IgG4 was significantly lower in the crescent group than in the control group. Considering the difference in complement-activating ability between IgG2 and IgG4 (IgG2 activates the complement system, while IgG4 does not [5]), our findings suggest that IgG2 deposits along GBM in the crescent group may have a direct role in crescent formation by facilitating the complement-associated inflammatory response. Our results also suggest that the crescent group and the control group may result from different immunological mechanisms, considering that a predominant glomerular deposition of IgG4 is characteristic of I-MN [6].

Arrizabalaga et al. [16] performed monoclonal antibody analysis of renal biopsy specimens from 2 MN patients with crescents, lacking evidence of S-MN, anti-GBM antibodies, and ANCA. They showed marked glomerular and interstitial infiltration of T cell subsets and macrophages, and abnormal expression of ICAM-1 antigens on proximal tubular epithelial cells, and suggested that proximal tubular epithelial cells may have played a role in local cell immune interactions in their patients. Indeed, moderate-to-severe tubulointerstitial lesions were more common in the crescent group than in the control group in Wang's cohort [20], Qian's cohort [21], and our cohort.

Table 9 summarizes the treatments and renal outcomes. In the previous cohort studies [19–21], mean or median follow-up periods were short (22.0–23.0 months). In Rodriguez's cohort [19], 21% of patients progressed to ESRD. In Wang's cohort [20], 32% of patients did not achieve CR or PR, and 11% of patients progressed to ESRD. In Qian's cohort [21], the median levels of eGFR at the last follow-up in the crescent group was 86.3 mL/min/1.73 m^2. Based on this result, they suggested a favorable short-term prognosis of patients in the crescent group. In the crescent group in our cohort (mean follow-up period, 79.0 months), 88% of patients were treated with steroids and 69% of patients were treated with steroids plus immunosuppressive agents. All patients achieved CR or PR. Although there was no patient who progressed to ESRD, doubling of serum Cr during follow-up was frequently observed. Higher proportions of crescent formation and interstitial fibrosis were significant risk factors for doubling of serum Cr.

In conclusion, clinicopathological and long-term prognostic features in the crescent group in our Japanese cohort, as compared with the control group, were as follows: (1) male predominance; (2) decreased eGFR at presentation; (3) moderate-to-severe tubulointerstitial lesions; (4) different distribution patterns of glomerular IgG-subclass depositions; and (5) frequently observed doubling of serum Cr during follow-up. Our findings

Table 9 Cases of MN with crescents without anti-GBM Ab and ANCA

References	Year	Patient no.	Treatment		Follow-up period (months)	Outcomes					
			PSL no. (%)	I-S no. (%)		CR no. (%)	PR no. (%)	NR no. (%)	Improvement	ESRD no. (%)	Unknown no. (%)
Kwan [14]	1991	1	1	1	2	ND	ND	ND		1	
Tse [15]	1997	3	3	3	7/36/84	ND	ND	ND	2/3	1	
Arrizabalaga [16]	1998	2	2 (100)	2 (100)	2/1	0	0	1	1/2	1	
Unver [17]	2008	1	1	1	4	ND	ND	ND	1/1		
Gadonski [18]	2010	1	1		ND	ND	ND	ND	0/1	1	
Rodriguez [19]	2014	19	14 (73.7)	12 (63.2)	22.0 (1.5–138.0)	1 (5.3)	8 (42.1)	3 (15.8)		4 (21.1)	3 (15.8)
Wang [20]	2015	28	12 (42.9)		22.7 ± 19.5	2 (7.1)	17 (60.7)	9 (32.2)		3 (10.7)	
Qian [21]	2016	58	55 (94.8)[a]		23.0 (1.0–70.0)	ND	ND	ND		1 (1.8)	3 (5.2)
Present study		16	14 (87.5)	11 (68.8)	79.0 (24.0–290.0)	10 (62.5)	6 (37.5)	0		0	

ANCA anti-neutrophil cytoplasmic antibodies, *CR* complete remission, *ESRD* end-stage renal disease, *anti-GBM Ab* anti-glomerular basement membrane antibodies, *I-S* immunosuppressive agents, *MN* membranous nephropathy, *ND* no data, *NR* no remission, *PR* partial remission, *PSL* prednisolone

[a] Details were unavailable

suggest that MN with crescents, in the absence of known clinical and immunological factors, should be regarded as a distinct variant of MN.

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Compliance with ethical standards

Disclosure All the authors have declared no competing interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee at which the studies were conducted (IRB approval number 1026) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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