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ORIGINAL ARTICLE

Clinicopathological characteristics of M-type phospholipase A2 receptor (PLA2R)-related membranous nephropathy in Japanese

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Abstract
Background Recent studies have suggested that assessments of serum antibodies against M-type phospholipase A2 receptor (PLA2R) and the glomerular expression of PLA2R antigen in biopsy specimens are useful for the diagnosis of primary membranous nephropathy (MN). In this study, we assessed both of them and investigated the clinicopathological characteristics of PLA2R-related Japanese MN.
Methods We retrospectively enrolled 22 primary and 3 secondary Japanese patients whose serum samples and renal specimens were collected before treatment. According to the findings of serum antibodies and antigen in glomeruli, the primary MN patients were classified into PLA2R-related or -unrelated MN. We compared their clinicopathological findings, including IgG subclass staining, and electron microscopic findings, and evaluated the predictors of proteinuria remission.
Results In primary MN, 16 patients (73 %) were classified into the PLA2R-related group, and 6 patients into the PLA2R-unrelated group. There was no significant difference in baseline laboratory data and electron microscopic findings, except for eGFR and serum IgG levels. IgG4-dominant deposition was more common in the related group (63 vs. 0 %). The 10 PLA2R-related patients with dominant IgG4 deposition had a lower rate and prolonged time in remission compared with the 6 PLA2R-related patients with non-dominant IgG4 (log-rank, $p = 0.032$). Furthermore, dominant IgG4 deposition was an unfavorable predictor of remission by multivariable Cox proportional hazard analysis.
Conclusions Assessments of both serum PLA2R antibodies and PLA2R antigen in glomeruli were more sensitive for the diagnosis of PLA2R-related MN, and among affected Japanese patients, those with dominant IgG4 deposition had worse clinical outcomes.

Keywords Electron microscopic findings · IgG subclass staining · Membranous nephropathy · Phospholipase A2 receptor · Western blotting

Introduction

Membranous nephropathy (MN) is the most common cause of nephrotic syndrome in adults. MN is characterized by immune complex deposition in the subepithelial space of glomerular capillaries. In 10–20 % of patients, MN is associated with an underlying disease, such as autoimmune disease (e.g., systemic lupus erythematosus), infection, drugs and malignancies [1]. When no underlying cause is identified, the disease is classified as primary (idiopathic). It has long been suspected that primary MN is evoked by the in situ formation of immune complexes as circulating antibodies react with a podocyte antigen. In 2009, M-type phospholipase A2 receptor (PLA2R) was identified as a target podocyte antigen in primary MN. Circulating

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autoantibodies against PLA2R were found in 70 % of patients with idiopathic MN, but not in those with secondary MN or other glomerular diseases [2]. The level of circulating anti-PLA2R autoantibody correlated with clinical disease activity and was useful to monitor response to treatment [3–5]. In addition, assessment of the glomerular expression of PLA2R antigen in biopsy specimens was also useful for the diagnosis of primary MN [6, 7]. Svobodova et al. [7] showed that assessment of both circulating anti-PLA2R antibodies and PLA2R antigen in biopsy might better categorize patients into different groups than only assessing anti-PLA2R antibodies, even during active disease.

In this study, we assessed both serum anti-PLA2R antibodies and PLA2R antigen in glomeruli at the same time before treatment and defined the patients with either elevated anti-PLA2R antibody in the serum or enhanced PLA2R in glomeruli as having PLA2R-related MN. We assessed their clinicopathological characteristics and clinical outcomes in comparison with those in PLA2R-unrelated primary MN and secondary MN.

Materials and methods

Patients

We retrospectively enrolled 25 Japanese MN patients who were admitted to Kanazawa Medical University Hospital between 1998 and 2013, and whose serum samples were collected before treatment at the time of renal biopsy. We followed these patients for at least 6 months (median (IQR), 26 [16.8–35.3] months). Diagnosis was confirmed in all patients by percutaneous needle renal biopsy. To detect secondary causes of MN, clinical workup including detailed medical history and physical examination, serological analysis (tumor markers, lupus autoantibodies, hepatitis B and C) and CT scan were conducted. The patients with lupus nephritis were excluded from this study.

In primary MN, we defined the patients who were either serum anti-PLA2R autoantibody- or glomerular PLA2R-positive as the PLA2R-related group and those who were negative in both serum and glomeruli as the PLA2R-unrelated group. Between these two groups, we compared the baseline clinicolaboratory data, pathological characteristics and clinical outcomes, such as remission rate.

We measured urine protein:creatinine ratio (uPCR) and evaluated clinical status according to Japanese clinical categories as follows: the nephrotic state, that is, the presence of heavy proteinuria (greater than 3.5 g/gCre) and hypoalbuminemia (less than 3.0 g/dL); incomplete remission type II, that is, mean daily proteinuria of 1.0–3.5 g/gCre accompanied by an improvement of serum albumin levels (more than 3.0 g/dL); incomplete remission type I, that is, mean daily proteinuria of 0.3–1.0 g/gCre with normal serum albumin levels (more than 3.0 g/dL); and complete remission, that is, daily proteinuria of less than 0.3 g/gCre with normal serum albumin levels. For the purpose of analysis, we defined remission as improvement of clinical status to the point of incomplete remission type I, according to the criterion of good long-term renal outcome in Japan [8]. The patients were treated non-randomly, depending on the judgment of the doctor in charge of each case. The protocol of this study was approved by the Clinical Study Ethics Review Board of Kanazawa Medical University. Prior to the study, verbal/written informed consent was obtained from all patients (Clinical Study Ethics Review Board of Kanazawa Medical University, Approval No. 80). This study was conducted according to the principles of the Declaration of Helsinki.

Western blotting

The anti-PLA2R autoantibodies in the serum were detected by Western blot analysis with cell lysate of HEK293 cells expressing recombinant PLA2R proteins. HEK293 cells were transfected with coding cDNA of the extracellular domain of PLA2R and homogenized in RIPA buffer (Wako Pure Chemicals, Japan) with protease inhibitor cocktail (Roche Applied Science, USA). Then, the cell lysate was mixed with 4× LDS sample buffer (Invitrogen, USA) and heated for 10 min at 70 °C. Equal amounts of the cell lysate were electrophoresed in 4–12 % Bis–Tris polyacrylamide gel (Invitrogen, USA) under non-reducing conditions and transferred to polyvinylidene difluoride membranes (Invitrogen, USA). The membranes were blocked with Blocking-One (Nacalai Tesque, Japan) and incubated with the serum at a dilution of 1:100. After washing, the membranes were incubated with horseradish peroxidase (HRP)-conjugated mouse anti-human IgG secondary antibody (Abcam) at a dilution of 1:20,000. 5 % Blocking-One in phosphate-buffered saline with 0.05 % Tween 20 (PBST) was used for all primary and secondary antibody dilutions. The immunoreactive bands were visualized by a chemiluminescent technique using ImmunoStar LD (Wako Pure Chemicals, Japan) as an HRP substrate.

Immunohistological staining

Fresh tissue specimens, embedded in OCT compound and frozen in acetone-dry ice mixture, were cut at a thickness of 3 μm on a cryostat. The frozen sections were fixed in 1:1 acetone:methanol and blocked with 10 % goat serum in 0.01 mol/L phosphate-buffered saline (PBS). To detect PLA2R, we used rabbit anti-PLA2R antibodies (Atlas Antibodies) at a dilution of 1:400, followed by fluorescein

isothiocyanate (FITC)-labeled goat anti-rabbit IgG antibodies (Cosmo Bio) at a dilution of 1:640. We detected the IgG subclass in the MN immune deposits with mouse anti-IgG1, -IgG2, -IgG3 and -IgG4 monoclonal antibodies (AbD Serotec), each at a dilution of 1:50, followed by FITC-labeled goat anti-mouse IgG antibodies (Cosmo Bio) at a dilution of 1:160. Immunofluorescence staining intensity was arbitrarily graded on a scale from 0 to 3 (0 negative, 1 weak staining, 2 moderate staining, 3 strong staining) and the median score was calculated (Supplementary Figures S1 and S2).

Electron microscopic examination

The specimens obtained from 24 patients were fixed with glutaraldehyde and osmium tetroxide, embedded in Epon 812, cut into 0.1 μm sections, double-stained with uranyl acetate and lead citrate, and examined with a Hitachi H-7650 electron microscope. For the electron microscopic study, we examined the specimens with emphasis on the phase and synchronicity of subepithelial and intramembranous electron-dense deposits and divided them into two subtypes, namely, homogeneous type with monophasic deposits and heterogeneous type with polyphasic deposits, as previously reported [9, 10]. We also used the Ehrenreich and Churg classification for the ultrastructural staging of MN [11].

Statistical analysis

Continuous measures and ranking scales were summarized using medians (25–75 % interquartile range), whereas categorical measures were summarized using counts and percentages. Mann–Whitney *U* test was used to assess the differences between two groups. Fisher's exact test was utilized to compare proportions. The Kaplan–Meier life-table method and Cox proportional hazard analysis were performed to evaluate predictors of remission of proteinuria. In addition, Cox proportional hazard models were developed with relevance to remission with subsequent addition of sociodemographic (age and sex), clinical variables (eGFR and uPCR), pathological findings (PLA2R, IgG subclass staining and electron microscopic stage), and treatment. *P* values of less than 0.05 were considered statistically significant. The Cox proportional hazard analysis was conducted using Stat Flex, version 6.0 (Artech, Japan), and the others were conducted using SPSS, version 20.0 (Chicago, IL, USA).

Results

PLA2R of primary and secondary MN in Japanese

In this study, 22 primary MN patients and 3 secondary MN patients were enrolled. The causes of secondary MN were malignancy (malignant thymoma, $n = 1$), bucillamine ($n = 1$) and chronic graft versus host disease (GVHD) after peripheral blood stem cell transplantation ($n = 1$).

In secondary MN, no patient had autoantibody in serum and glomerular PLA2R deposits. On the other hand, in 12 primary MN patients (55 %), circulating PLA2R autoantibodies were positive with glomerular PLA2R in 10 patients, but not in 2 patients. Of the 10 primary MN patients with no detectable PLA2R autoantibodies in serum, 4 patients had glomerular PLA2R deposits. According to these findings, 16 patients (73 %) were classified into the PLA2R-related group and 6 patients into the PLA2R-unrelated group.

Clinicopathological characteristics of primary MN related to PLA2R positivity

The clinicolaboratory data and pathological characteristics at baseline are presented in Table 1. There was no significant difference in clinicolaboratory data, except for eGFR and serum IgG levels. The intensity of IgG4 deposits was much higher in the PLA2R-related group (3.0 vs. 0.5, $P = 0.010$). Then, IgG4 pre- or co-dominant patients were more common in the PLA2R-related group (63 vs. 0 %, $P = 0.0152$). There was no significant difference in the electron microscopic findings.

Clinical outcomes of primary MN

The primary MN patients were treated using supportive therapy ($n = 11$, 50 %), corticosteroid alone ($n = 2$, 9.1 %), cyclosporine with steroid ($n = 6$, 27.3 %) or mizoribine with steroid ($n = 3$, 13.6 %). During the follow-up period, no patient developed end-stage renal failure (ESRF). The results of the univariate time-dependent analysis by the Kaplan–Meier method are shown in Fig. 1. Although there was no significant difference in the remission rate and the time to remission between the PLA2R-related and PLA2R-unrelated groups (Fig. 1a), the 10 PLA2R-related patients with dominant IgG4 deposition had a low rate and prolonged time in remission compared with the 6 PLA2R-related patients with non-dominant IgG4 (log-rank, $p = 0.032$, Fig. 1b). The clinical predictors of remission in the multivariable Cox proportional hazard analysis are presented in Table 2. It is revealed that dominant IgG4 deposition was an unfavorable predictor and immunosuppressive therapy was a favorable predictor of remission.

In patients whose serum antibodies were positive at baseline, with the exception of one patient, we assessed this variable again at the end of the follow-up period (Table 3 and Supplementary Figure S3). In 4 of 8 patients (50 %) who achieved remission, the serum antibodies became

Table 1 Clinicolaboratory data and pathological characteristics of primary MN at baseline

	PLA2R-related [$n = 16$]	PLA2R-unrelated [$n = 6$]	P value
Age (years)	64.5 [61–70]	70 [64–74]	0.336
Male/female	12/4	4/2	0.541
Follow-up (months)	26 [13–36]	24 [16–34]	0.914
Serum creatinine (mg/dl)	0.77 [0.65–0.89]	0.96 [0.80–1.41]	0.083
eGFR (ml/min/1.73 m^2)	75.2 [64.7–82.3]	59.3 [28.9–68.6]	0.049*
Serum albumin (g/dl)	2.35 [2.05–3.05]	2.70 [1.90–3.40]	0.684
Serum IgG (mg/dl)	691.5 [517.5–989.5]	1,045.0 [1,038.0–1,079.0]	0.018*
uPCR (g/gCre)	10.0 [5.3–12.8]	5.5 [3.7–8.1]	0.261
Immunohistological staining			
IgG1 score	1.5 [0.0–2.5]	1.5 [1.0–2.0]	0.909
IgG2 score	0.0 [0.0–1.0]	0.0 [0.0–0.0]	0.089
IgG3 score	1.0 [0.0–1.5]	0.0 [0.0–1.0]	0.448
IgG4 score	3.0 [1.0–3.0]	0.5 [0.0–1.0]	0.010*
IgA score	0.0 [0.0–1.0]	0.0 [0.0–1.0]	0.763
C1q score	0.0 [0.0–1.0]	0.5 [0.0–1.0]	0.738
C3 score	3.0 [0.5–3.0]	1.0 [0.0–2.0]	0.143
Electron microscopic findings	PLA2R-related [$n = 15$]	PLA2R-unrelated [$n = 6$]	P value
Ehrenreich–Churg stage	2.0 [1.0–2.5]	1.5 [1.0–2.0]	0.480
Subtype homogeneous	14 [93 %]	5 [83 %]	0.500
Subtype heterogeneous	1 [7 %]	1 [17 %]	

PLA2R M-type phospholipase A2 receptor, *MN* membranous nephropathy, *uPCR* urine protein/creatinine ratio

* P values of less than 0.05

negative. On the other hand, in all patients who did not achieve remission, the serum antibodies were still positive.

Discussion

Although the exact mechanisms and the role of PLA2R in the pathogenesis of primary MN are currently unknown, PLA2R is probably one of the intrinsic glomerular antigens. In this study, we collected serum samples before treatment at the time of renal biopsy, and assessed both serum anti-PLA2R antibodies and glomerular PLA2R deposits. In previous studies, the prevalence of serum anti-PLA2R antibodies by western blotting in patients with primary MN ranged from 69 to 82 % [2, 4, 5, 12, 13] and the prevalence of glomerular PLA2R deposits ranged from 69 to 84 % [6, 7, 14, 15]. In this study, we detected serum anti-PLA2R antibodies in 55 % and glomerular PLA2R deposits in 64 % of patients with primary MN. The reason for the low prevalence in our serum antibody detection is unclear at this time. A larger Japanese study ($n = 96$) by our colleagues showed similar results (50 %, unpublished data). In other Asian countries, on the other hand, the reported prevalence of serum anti-PLA2R antibodies was 82 % in a Chinese study [12] and 69 % in a Korean study [13]. In this study, combined with the results of glomerular PLA2R deposits, the sensitivity of the diagnosis of PLA2R-related MN rose to 73 %.

In previous studies, serum anti-PLA2R antibodies and PLA2R antigen in glomeruli were largely negative in patients with secondary causes of MN including lupus nephritis, HBV, and malignancy-associated MN [12, 15]. We also examined 3 patients with representative cause of secondary MN (1 malignancy, 1 GVHD, and 1 bucillamin) and found that no patient had autoantibody in serum and glomerular PLA2R deposits.

Similar to previous studies [7, 14], we found that 4 patients had no circulating antibodies, although they had PLA2R detected in glomeruli, while 2 patients with circulating antibodies had no detectable PLA2R in glomeruli. The findings of serum negativity and glomerular positivity were explained by the rapid clearance of antibodies from the blood or by the late sampling of patients when proteinuria persisted because of irreversible ultrastructural changes [7, 14]. However, there was no significant difference in proteinuria at baseline and time to remission between the patients with serum negativity and glomerular positivity and the patients with both serum and glomerular positivity in this study. On the other hand, for the patients with circulating antibodies but without detectable PLA2R in glomeruli, it was speculated that the antibodies were not nephritogenic or that epitopes were poorly accessible at the time of renal biopsy [7, 14]. In this study, circulating antibodies were not detected after remission in 2 patients with serum positivity and glomeruli negativity at baseline (Table 3). Finally, neither PLA2R antigen in glomeruli nor

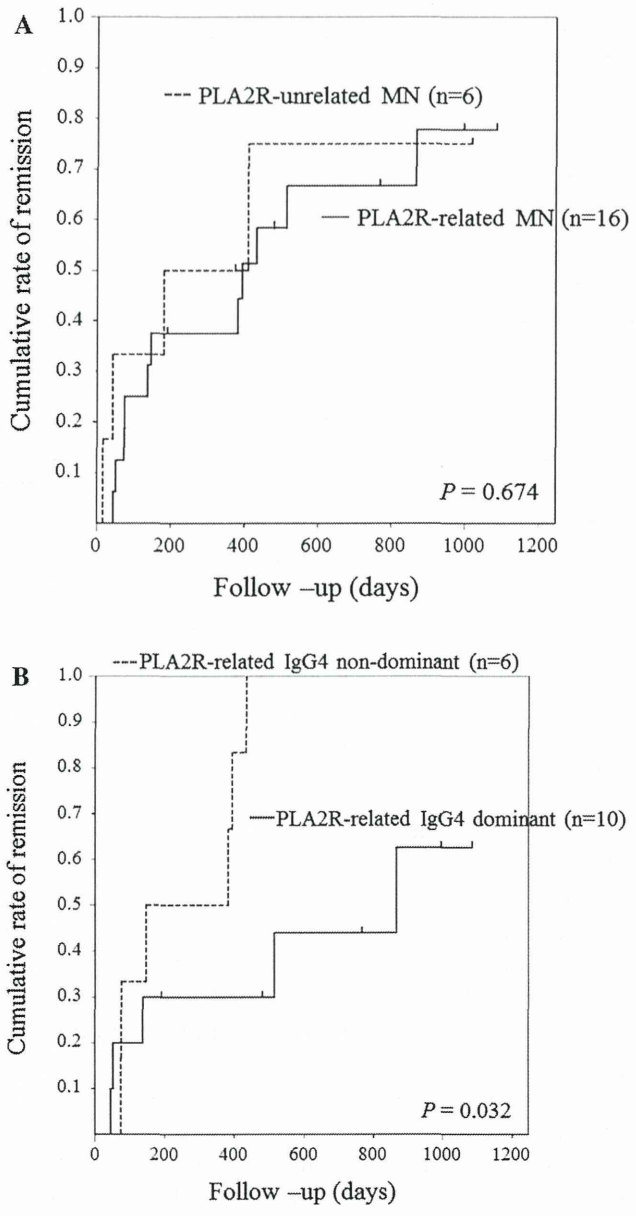

Fig. 1 Kaplan–Meier curves of remission in Japanese membranous nephropathy. **a** There was no significant difference in the remission rate and the time to remission between the PLA2R-related and -unrelated groups. **b** The 10 PLA2R-related patients with dominant IgG4 deposition had a low rate and prolonged time in remission compared with the 6 PLA2R-related patients with non-dominant IgG4 (log-rank, $p = 0.032$)

PLA2R antibody in serum was found in 6 primary MN patients. It is possible that they have an as yet undetected cause of MN or other recently described antigens, such as alpha-enolase, aldose reductase and SOD2 [16].

IgG subtype analysis in glomeruli has been carried out for the discrimination between primary (idiopathic) and secondary MN. IgG4-dominant staining is associated with primary MN, whereas IgG1, IgG2 and IgG3 dominate in the deposits of secondary MN [17–19]. Serum autoantibodies to PLA2R are largely IgG4, and IgG4 could be co-localized with the PLA2R antigen within the glomerular immune deposits of idiopathic MN [2, 3], except for some cases such as monoclonal IgG3-kappa targeting the PLA2R [20]. Consistent with these findings, in our PLA2R-related group, IgG4-dominant deposits in glomeruli were more common than in the PLA2R-unrelated group and secondary MN patients.

Furthermore, IgG4 dominancy was an unfavorable predictor of remission. Hofstra et al. [3] found that spontaneous remissions were less likely to occur in patients with high serum IgG4 antibody titers to PLA2R. Although IgG4 does not activate the classic complement pathway, there are usually abundant deposits of complement, including C4 and C3, in the immune deposits in primary MN. A recent preliminary study reported that degalactosylated anti-PLA2R IgG4 can bind mannan-binding lectin, which suggests the possibility that the lectin pathway of complement might be activated in glomerular IgG4-predominant immune deposits [21]. We speculated that two characteristics in the immune regulation of IgG4 were relevant to the worse remission of proteinuria. One is the finding that the production of IgG4 antibodies seems to be driven by T helper 2 (Th2) cytokine activation in primary MN [22]. The other is its tendency to appear only after prolonged immunization. It usually takes many months of repeated antigen exposure before IgG4 responses become prominent [23]. In this regard, Huang et al. [24] found, in early stages of MN, that IgG1 was the dominant IgG subclass, whereas IgG4 became dominant in later stages. We also found that almost all IgG4-dominant patients (8 of 10) had other IgG subclass deposits to some extent.

Then, we hypothesized that cases of PLA2R-related MN, if caused by immune reactions evoked by repeated exposure to the intrinsic antigen, would have later stages or the heterogeneous type with a different phase of electron-dense deposits. However, there was no significant difference in electron microscopic findings between the PLA2R-related and -unrelated groups. Moreover, early electron-dense deposits such as stage 1 including IgG4 were also observed in the IgG4-predominant or co-dominant group. These findings may suggest that co-localized non-complement-activating IgG4 antibodies and other IgG subclasses such as IgG1, 2 and 3 can augment complement-activating injury, as previously reported in a mouse antibody-mediated allograft rejection model [25].

We found that four patients whose serum antibodies were still positive even after they achieved remission. Among serum positive and negative patients after remission, there was no significant difference about treatment and IgG subclass staining. In previous studies, although there were few atypical patients [4, 7], it was found that anti-PLA2R antibodies disappeared in advance of

Table 2 The multivariate cox proportional hazard model for remission ($n = 22$)

Predictors	Model 1[a]			Model 2[b]		
	HR	95 % CI	*P* value	HR	95 % CI	*P* value
Age (per 5-year interval)	1.35	0.76–2.40	0.299	1.64	0.64–4.17	0.299
Female (vs. male)	2.76	0.75–10.1	0.125	6.02	0.49–73.9	0.160
eGFR (per 10 ml/min/1.73 m^2)	1.56	0.96–2.54	0.071	2.01	0.82–4.91	0.124
uPCR (per 1.0 g/gCre)	–	–	–	0.95	0.72–1.27	0.771
PLA2R-related (vs. -unrelated)	1.24	0.29–5.20	0.759	1.41	0.30–6.55	0.659
IgG4-dominant (vs. non-dominant)	0.08	0.01–0.51	0.007*	0.02	0.00–0.91	0.044*
Ehrenreich–Churg stage	–	–	–	0.74	0.21–2.60	0.643
Immunosuppressive therapy (vs. supportive therapy)	3.39	1.20–47.6	0.140	7.58	1.20–47.6	0.030*

HR hazard ratio, *CI* confidence interval, *PLA2R* M-type phospholipase A2 receptor, *uPCR* urine protein/creatinine ratio

[a] Model 1: adjusted for age, sex, eGFR, relevance to PLA2R, IgG4 dominancy, and treatment

[b] Model 2: model 1 with additional adjustment for uPCR and Ehrenreich–Churg stage

* *P* values of less than 0.05

Table 3 Change of serum anti-PLA2R antibody positivity in primary MN patients whose serum antibodies were positive at baseline ($n = 11$)

Patient	Baseline				Treatment	Remission	Follow-up	
	uPCR (g/gCre)	Serum PLA2R	Biopsy PLA2R	IgG4 dominancy			uPCR (g/gCre)	Serum PLA2R
A	3.21	(+)	(+)	Dominant	Supportive	Yes	0.16	(+)
B	19.31	(+)	(+)	Dominant	IS	Yes	0.05	(+)
C	5.82	(+)	(+)	Dominant	Supportive	Yes	0.06	(−)
D	4.7	(+)	(+)	Non-dominant	Supportive	Yes	0.45	(+)
E	9.88	(+)	(+)	Dominant	IS	Yes	0.18	(+)
F	2.15	(+)	(+)	Non-dominant	IS	Yes	0.25	(−)
G	7.82	(+)	(+)	Dominant	IS	No	10.12	(+)
H	12.38	(+)	(+)	Dominant	Supportive	No	8.07	(+)
I	6.84	(+)	(+)	Dominant	Supportive	No	5.79	(+)
J	21.34	(+)	(−)	Dominant	IS	Yes	0.15	(−)
K	10.19	(+)	(−)	Non-dominant	IS	Yes	0.03	(−)

PLA2R M-type phospholipase A2 receptor, *uPCR* urine protein/creatinine ratio, *IS* immunosuppressive therapy

remission of proteinuria [4, 5]. Furthermore, anti-PLA2R antibodies appeared prior to recurrence of MN in a transplant recipient [20]. In this study, the findings that serum antibodies still positive after remission of proteinuria might predict recurrence of proteinuria in the near future, or suggest that their antibodies lost nephritogenicity. For instance, it is possible that the antibodies changed their affinity or avidity, their subclass switched to non-complement-activating subclass or, in IgG4 antibodies, their degalactosylation was modified.

Finally, having PLA2R-related MN was not a predictor for remission of proteinuria in this study. However, Hoxha et al. [26] found that high anti-PLA2R antibody titer measured by ELISA was an independent risk factor for not achieving remission. For the purpose of evaluating the clinical outcome, such as remission of proteinuria, quantitative analysis by ELISA might be better than qualitative analysis by western blotting or assessing PLA2R in glomeruli.

In conclusion, assessments of both serum anti-PLA2R antibody and PLA2R antigen in glomeruli at the same time are a more sensitive method for the diagnosis of PLA2R-related primary MN at present. However, there are discrepancies between the presence of serum antibody and the detection of glomerular antigen. In further investigation, on a larger sample of patients, we need to classify PLA2R-related MN patients into three groups according to serum antibody positivity/negativity and glomerular antigen positivity/negativity, and compare their clinicopathological characteristics and clinical outcomes. As expected, IgG4 antibody is an important clue to elucidate the pathogenesis of PLA2R-related MN. Further investigations are necessary to resolve the issue of serum antibodies positivity after remission.

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Conflict of interest None of the authors has any competing interests.

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ORIGINAL ARTICLE

Outcomes of primary nephrotic syndrome in elderly Japanese: retrospective analysis of the Japan Renal Biopsy Registry (J-RBR)

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Abstract
Background and objectives There are very little data available regarding nephrotic syndrome (NS) in elderly (aged ≥65 years) Japanese. The aim of this study was to examine the causes and outcomes of NS in elderly patients who underwent renal biopsies between 2007 and 2010.
Design, setting, participants, and measurements From July 2007 to June 2010, all of the elderly (aged ≥65 years) Japanese primary NS patients who underwent native renal biopsies and were registered in the Japan renal biopsy registry (J-RBR; 438 patients including 226 males and 212 females) were identified. From this cohort, 61 patients [28 males and 33 females including 29, 19, 6, 4, and 3 patients with membranous nephropathy (MN), minimal change nephrotic syndrome (MCNS), focal segmental glomerulosclerosis (FSGS), membranoproliferative glomerulonephritis (MPGN), and other conditions, respectively] were registered from the representative multi-centers over all districts of Japan, and analyzed retrospectively. The treatment outcome was assessed using proteinuria-based criteria; i.e., complete remission (CR) was defined as urinary protein level of <0.3 g/day or g/g Cr, and incomplete remission type I (ICR-I) was defined as urinary protein level of <1.0–0.3 g/day or g/g Cr, and renal dysfunction was defined as a serum creatinine (Cr) level of 1.5 times the baseline level.
Results In this elderly primary NS cohort, MN was the most common histological type of NS (54.8 %), followed

Special report from the Japan Renal Biopsy Registry (J-RBR).
On behalf of the Committee for the Standardization of Renal Pathological Diagnosis and Renal Biopsy and Disease Registry of the Japanese Society of Nephrology.
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by MCNS (19.4 %), FSGS (17.4 %), and MPGN (8.4 %). Of the patients with MN, MCNS, or FSGS, immunosuppressive therapy involving oral prednisolone was performed in 25 MN patients (86.2 %), 18 MCNS patients (94.7 %), and all 6 FSGS patients (100 %). CR was achieved in all 19 (100 %) MCNS patients. In addition, CR and ICR-I were achieved in 16 (55.2 %) and 18 (62.1 %) MN patients and 4 (66.7 %) and 5 (83.3 %) FSGS patients, respectively. There were significant differences in the median time to CR among the MCNS, FSGS, and MN patients (median: 26 vs. 271 vs. 461 days, respectively, $p < 0.001$), and between the elderly (65–74 years, $n = 7$) and very elderly (aged ≥75 years, $n = 12$) MCNS patients (7 vs. 22 days, $p = 0.037$). Relapse occurred in two (6.9 %) of the MN and nine (47.4 %) of the MCNS patients. Renal dysfunction was observed in five (7.2 %) of the MN patients. Serious complications developed in eight (14.8 %) patients, i.e., two (3.7 %) patients died, four (7.4 %, including three MCNS patients) were hospitalized due to infectious disease, and two (3.7 %) developed malignancies. The initiation of diabetic therapy was necessary in 14 of the 61 patients (23.0 %) with much higher initial steroid dosage.

Conclusion Renal biopsy is a valuable diagnostic tool for elderly Japanese NS patients. In this study, most of elderly primary NS patients respond to immunosuppressive therapy with favorable clinical outcomes. On the other hand, infectious disease is a harmful complication among elderly NS patients, especially those with MCNS. In future, modified clinical guidelines for elderly NS patients should be developed.

Keywords Nephrotic syndrome · Elderly · Japanese · Outcome · Immunosuppressive therapy · Complication

Introduction

In Japan, elderly individuals; i.e., those aged 65 and over, accounted for 25.8 % of the total population in October 2010, and this will increase to 30.5 % by 2025 [1]. As life expectancy increases, more elderly patients with chronic renal diseases are surviving longer. In addition, the progressive decline in the glomerular filtration rate that occurs with age and age-related systemic diseases, such as anti-neutrophil cytoplasmic antibody (ANCA)-positive vasculitis, are expected to contribute to an increased incidence of renal disease in the elderly population [2].

As for nephrotic syndrome (NS), a previous study demonstrated that the elderly accounted for 1160 of the 2753 NS patients (42.4 %) registered in Japan. In addition, NS was found to be the most common indication for renal biopsy among both the elderly (aged ≥65 years, 36.3 %) and very elderly (aged ≥75 years, 50.7 %). Furthermore, membranous nephropathy (MN) was the most common pathological type of NS among the elderly (*n* 365, 31.5 %) and very elderly (*n* 45, 28.1 %), followed by minimal change nephrotic syndrome (MCNS; *n* 146, 12.6 %; *n* 19, 11.9 %) and focal segmental glomerulosclerosis (FSGS; *n* 68, 5.9 %; *n* 12, 7.5 %) [2].

Several studies involving limited numbers of elderly NS Japanese patients have reported that renal biopsy can provide significant diagnostic and prognostic information [3–7]. However, regarding the available therapies for and outcomes of elderly NS patients (and analyses of these factors in patients aged over 75 years) only single-center studies from Japan and Hong Kong and a study based on the Spanish Registry of Glomerulonephritis have been reported [7–9].

In 2007, the Committee for the Standardization of Renal Pathological Diagnosis and the Working Group for the Renal

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Table 1 Background data of the elderly nephrotic syndrome cohort in the J-RBR (2007–2010)

Primary nephrotic diseases	Cases (%)	Age at renal biopsy [median (range)]
Subjects (male: female = 226: 212)	438	73 (65–88)
Membranous nephropathy	240 (54.8)	73 (65–88)
Minimal change nephrotic syndrome	85 (19.4)	74 (65–85)
Focal segmental glomerulosclerosis	45 (10.3)	74 (65–83)
Membranoproliferative glomerulonephritis (type I or III)	37 (8.4)	73 (65–84)
Mesangial proliferative glomerulonephritis	12 (2.7)	75 (65–87)
Crescentic glomerulonephritis	9 (2.1)	73 (65–84)
Endocapillary proliferative glomerulonephritis	6 (1.4)	73 (65–87)
Sclerosing glomerulonephritis	1 (0.2)	77
Others	3 (0.7)	71 (66–77)

J-RBR Japan Renal Biopsy Registry

Biopsy Database of the Japanese Society of Nephrology established the first nationwide, web-based, prospective registry system, the Japan Renal Biopsy Registry (J-RBR), to record pathological, clinical, and laboratory data about the renal biopsies performed in Japan [10]. This nationwide registry system can be used to facilitate national epidemiological studies of renal diseases such as NS.

The aim of this study was to retrospectively examine the outcomes of NS patients using a large group of elderly (65–74 years) and very elderly (75 years old or older) patients who had undergone native renal biopsy and were scheduled to be followed up for 5 years (median follow-up period: approx. 2 years).

Materials and methods

J-RBR system and subjects

The researchers of the Committee for the Standardization of Renal Pathological Diagnosis and the Working Group for the Renal Biopsy Database of the Japanese Society of Nephrology set up the J-RBR [10]. From July 2007 to June 2010, all of the elderly (aged ≥65 years) and very elderly (aged ≥75 years) primary NS patients who had undergone native renal biopsy and been registered in the J-RBR (438 patients including 226 males and 212 females) were identified (Table 1). From this cohort, 61 patients [28 males and 33 females including 29, 19, 6, 4, and 3 patients with MN, MCNS, FSGS, membranoproliferative glomerulonephritis (MPGN), and other conditions, respectively] were registered from the representative multi-centers over all districts of Japan, and their data were analyzed retrospectively (Table 2, Chi-square value for background cohort vs. selected cohort: 4.497, $p = 0.4803$). The patients in this study showed the nephrotic range proteinuria at least once before renal biopsy. Patient data including information regarding each patient's age, gender, and laboratory findings as well as the clinical category and pathological diagnosis of their condition were electronically recorded at each institution and registered on the J-RBR webpage via the Internet Data and Information Center for Medical Research (INDICE) system, which is part of the University Hospital Medical Information Network (UMIN). Clinical data, including urinalysis data; daily proteinuria values;

Table 2 Data for the 61 subjects from 10 centers selected for this retrospective study

Primary nephrotic diseases	Cases (%)	Gender (male:female)	Age at renal biopsy [Median (range)]
Subjects	61	28:33	73 (65–86)
Membranous nephropathy	29 (47.5)	12:17	72 (66–82)
Minimal change nephrotic syndrome	19 (31.1)	7:12	76 (65–86)
Focal segmental glomerulosclerosis	6 (9.8)	4:2	75 (70–81)
Membranoproliferative glomerulonephritis (type I or III)	4 (6.6)	2:2	73 (66–76)
Crescentic glomerulonephritis	2 (3.3)	2:0	71 (66–76)
Endocapillary proliferative glomerulonephritis	1 (1.6)	1:0	65 (65)

There was no significant difference between the data for the background cohort and those for the selected subjects (Chi-square 4.497, $p = 0.4803$)

Registered centers and cases: Okayama Univ.: 17 cases; Niigata Univ.: 9 cases; Fukuoka Univ.: 7 cases, Tsukuba Univ.: 6 cases; Kanazawa Med Univ.: 5 cases; Miyazaki Univ. & Hokkaido Univ.: 4 cases each; Fujita Health Univ., Kurume Univ., & Shizuoka Prefectural Hospital: 3 cases each