and laboratory data such as serum creatinine (Cr), total protein (TP), albumin, total cholesterol (TC), low-density lipid cholesterol (LDL-C), high-density lipid cholesterol (HDL-C), glycated hemoglobin (HbA1c), and hemoglobin levels were also recorded on a check sheet at the time of the renal biopsy and at 1, 3, 6, 12, and 24 months after J-RBR registration.

The ethical committee of the Japanese Society of Nephrology comprehensively examined and approved the study protocol, and the local committees of the participating centers and their affiliate hospitals individually approved the study. The J-RBR is registered at the Clinical Trial Registry of UMIN (UMIN000000618).

Clinical categories and pathological diagnoses

Glomerular disease was classified into the following clinical categories: NS, chronic nephritic syndrome, recurrent or persistent hematuria, acute nephritic syndrome, and rapidly progressive nephritic syndrome, based on the criteria developed by the WHO [11]. NS was defined as proteinuria of $\geq$3.5 g/day and/or a urinary protein/creatinine ratio (UPCR) of $\geq$3.5 g/g Cr combined with hypoalbuminemia (serum albumin <3.0 g/dl) and/or hypoproteinemia (total protein <6.0 g/dl), as defined by the Progressive Renal Diseases Research (2011) criteria [12].

The patients' renal histological diagnoses were classified according to their pathogeneses (group A) or histopathological findings (group B): group A: primary glomerular disease (except IgA nephropathy, IgAN), IgAN, purpura nephritis, lupus nephritis, myeloperoxidase- antineutrophil cytoplasmic antibody (ANCA)-positive nephritis, protein 3-ANCA-positive nephritis, anti-glomerular basement membrane antibody nephritis, hypertensive nephrosclerosis, thrombotic microangiopathy, diabetic nephropathy, amyloid nephropathy, Alport syndrome, thin basement membrane disease, infection-related nephropathy, kidney transplantation, and others; group B: minor glomerular abnormalities, FSGS, MN, endocapillary proliferative glomerulonephritis, MPGN (types I and III), and crescentic and necrotizing glomerulonephritis [11].

Therapy for nephrotic syndrome and treatment response

Due to the retrospective nature of this study, the immunosuppressive or supportive therapy protocols varied between individual centers. The use of immunosuppressant or supportive drugs was checked on the record sheet and registered in the web-based folder for this study.

The response of NS to treatment was assessed according to the definition outlined by the Japanese Society of Nephrology [12], i.e., a urinary protein level of <0.3 /day or a UPCR of <0.3 g/g Cr, a urinary protein level of between 0.3 and <1.0 g/day or a UPCR of between 0.3 and <1.0 g/g Cr, a urinary protein level of between 1.0 and <3.5 g/day or a UPCR of between 1.0 and <3.5 g/g Cr, and a urinary protein level of $\geq$3.5 g/day or a UPCR of $\geq$5 g/g Cr were defined as complete remission (CR), incomplete remission (ICR)-I, ICR-II, and ineffective, respectively. The clinical significance to achieve ICR-I in nephrotic Japanese was previously reported [19, 20].

The responses of CR, ICR-I and ICR-II were judged by the recorded time from the initiation of immunosuppressive therapy or biopsied time, if patients were not treated by immunosuppressive therapy, to the first remission of proteinuria. When the patient's urinary protein level did not decrease to <1 g/day and/or their UPCR did not fall to <1.0 g/g Cr after 6 months' treatment, e.g., with steroids and/or immunosuppressive drugs, their condition was defined as intractable NS.

Relapse was defined as daily proteinuria of >1.0 g, a UPCR of >1.0 g/g Cr, and/or a dipstick urinary protein value of $\geq$1+ after CR had been achieved.

Renal dysfunction was defined as a serum Cr level of 1.5 times the initial baseline level.

Statistical analyses

Continuous variables are reported as mean values (standard deviation, SD). Statistical analyses were performed using SPSS version 18.0 (SPSS, Tokyo, Japan). Comparisons of categorical variables among groups of different indications or diagnoses were performed using Fischer's exact test. Continuous variables were compared using the Student's *t* test or the ANOVA test for parametric data and Wilcoxon signed-rank test or the Kruskal–Wallis test for non-parametric data. The cumulative probability of a first remission was calculated using the Kaplan–Meier method. The effectiveness of immunosuppressive therapy and the influence of age on the treatment outcome were compared using the log-rank test and multivariate Cox regression analysis. *P* values of <0.05 (obtained by two-tailed testing) were considered to indicate statistical significance.

Results

The baseline background data of the patients with the 3 major pathological types of primary NS (MCNS, MN, and FSGS) are shown in Table 3. The MCNS patients exhibited significantly lower systolic blood pressure (mean: 118.9 $\pm$ SD 16.0, 133.2 $\pm$ 19.6 for MN, 136.5 $\pm$ 11.7 for FSGS mmHg, $p = 0.010$) and significantly higher serum TC levels than the MN and FSGS patients (394.4 $\pm$ 104.3 for MCNS, 293.8 $\pm$ 113.7 for MN, 295.7 $\pm$ 112.5 for FSGS mg/dL, $p = 0.027$). On the other hand, the FSGS

Table 3 Demographics of the elderly patients with the 3 major pathological types of NS

Cases Elderly/very elderly (n)	MN (29 cases) (17:12)		MCNS (19 cases) (7:13)		FSGS (6 cases) (2:4)		*P* value
	Mean	SD	Mean	SD	Mean	SD	
Height (cm)	153.7	9.2	154.4	8.2	153.3	7.5	0.938
Weight (kg)	58.4	7.8	62.9	10.6	61.2	10.9	0.286
Systolic BP (mmHg)	133.2	19.6	118.9[a]	16.0	136.5	11.7	0.010
Diastolic BP (mmHg)	77.6	14.2	72.2	6.8	67.2	7.5	0.104
Edema (%)	26 cases (89.7 %)		18 cases (94.7 %)		6 cases (100 %)		0.615
Daily proteinuria (g)	4.56	2.16	5.47	3.56	8.90[b]	3.50	0.032
uPCR (g/g Cr)	6.86	3.96	7.20	4.19	7.56	3.55	0.792
Hematuria (> 1 +)	20 cases (69.0 %)		9 cases (47.4 %)		4 cases (66.7 %)		0.310
Serum Cr (mg/dL)	0.94	0.44	1.17	0.67	2.37[b]	1.34	0.004
Serum BUN (mg/dL)	14.7	6.9	20.4	12.3	30.2[b]	7.8	0.004
Serum TP (g/dL)	4.79	0.86	4.64	0.40	4.97	0.74	0.762
Serum Alb (g/dL)	1.97	0.56	1.65	0.38	2.05	0.48	0.063
Serum TC (mg/dL)	293.8	113.7	394.4[a]	104.3	295.7	112.5	0.027
Serum LDL-C (mg/dL)	208.0	54.2	270.8	83.9	263.0	158.4	0.140
Serum HDL-C (mg/dL)	58.8	16.4	66.1	19.2	63.8	15.3	0.427
TG (mg/dL)	252.8	107.7	222.7	93.8	204.3	151.7	0.267
HbA1c (%)	5.28	0.54	5.68	0.31	5.40	0.25	0.076
Hb (g/dL)	12.55	1.46	12.54	1.85	10.88	1.48	0.082
IgG levels (mg/dL) (*n*: 26/16/4)	705	254	741	318	953	180	0.196

P values are for comparisons among the three histological types, i.e., MN, MCNS, and FSGS

MN membranous nephropathy, *MCNS* minimal change nephrotic syndrome, *FSGS* focal segmental glomerulosclerosis, *BP* blood pressure, *uPCR* urinary protein to creatinine ratio, *Cr* creatinine, *BUN* blood urea nitrogen, *TP* total protein, *Alb* albumin, *TC* total cholesterol, *LDL-C* low-density lipid cholesterol, *HDL-C* high-density lipid cholesterol, *TG* triglycerides, *HbA1c* glycated hemoglobin, *Hb* hemoglobin

[a] the value for MCNS was significantly different from those for MN and FSGS

[b] the value for FSGS was significantly different from those for MN and MCNS

patients exhibited significantly increased daily proteinuria (8.90 ± 3.50 for FSGS, 4.56 ± 2.16 for MN, 5.47 ± 3.56 for MCNS g/day, $p = 0.032$), serum creatinine levels (2.37 ± 1.34 for FSGS, 0.94 ± 0.44 for MN, 1.17 ± 0.67 for MCNS mg/dL, $p = 0.004$), and serum blood urea nitrogen levels (30.2 ± 7.8 for FSGS, 14.7 ± 6.9 for MN, 20.4 ± 12.3 for MCNS mg/dL, $p = 0.004$) compared with the other groups. No significant difference in the serum TP level or serum albumin level was detected among the various forms of NS. In addition, there was no difference in the frequency of hematuria (>1+) among the various types of NS because about half of the MCNS patients (47.4 %) had hematuria.

Initial immunosuppressive therapy for elderly NS patients in Japan

Among the three major pathological types of primary NS (MN, MCNS, and FSGS), oral prednisolone (PSL) was administered as an immunosuppressive therapy to 25 MN (86.2 %) patients, 18 MCNS (94.7 %) patients, and all 6 FSGS (100 %) patients, as shown in Table 4. In total, 49 patients (90.7 %) received oral PSL. In addition, intravenous methylprednisolone (mPSL) therapy, cyclosporine (CyA), oral cyclophosphamide, mizoribine, and mycophenolate mofetil were administered to 6 (11.1 %), 23 (42.6 %), 2 (3.7 %), 1 (1.9 %), and 1 (1.9 %) patient(s), respectively. Regarding the immunosuppressive drugs used to treat each pathological type of NS, intravenous mPSL therapy was most frequently used to treat FSGS (50 %), whereas CyA was most commonly used to treat MN and FSGS (58.6 and 50.0 %, respectively).

Among the MPGN patients, three (75 %) were treated with oral PSL and intravenous mPSL, and cyclophosphamide and mizoribine were used in one case each. The remaining MPGN patient dropped out after undergoing a renal biopsy and so did not receive immunosuppressive therapy. The two patients with crescentic glomerulonephritis (CrGN) were treated with oral PSL with or without intravenous mPSL, and the patient with endocapillary

Table 4 Clinical outcome of elderly patients with the 3 major pathological types of NS

Subjects [Elderly/very elderly]	MN (*n*:29) [17/12]		MCNS (*n*:19) [7/12]		FSGS (*n*:6) [2/4]		Total (*n*:54) [26/28]	
Follow-up period (days)	578		701		767		718	
Median [interquartile range]	[404–970]		[318–701]		[423–839]		[395–916]	
Outcomes	n	%	n	%	n	%	n	%
ICR type II (UP 1.0 to < 3.5 g/day)	27	93.1	19	100	5	83.3	51	94.4
Elderly	16	94.1	7	100	2	100	25	96.2
Very elderly	11	91.7	12	100	3	75.0	26	92.9
ICR type I (UP 0.3 to < 1.0 g/day)	18	62.1	19	100	5	83.3	42	77.8
Elderly	10	58.8	7	100	2	100	19	73.1
Very elderly	8	66.7	12	100	3	75.0	23	82.1
Complete remission (UP < 0.3 g/day)	16	55.2	19	100	4	66.7	39	72.2
Elderly	9	52.9	7	100	1	50.0	17	65.4
Very elderly	7	58.3	12	100	3	75.0	22	78.6
Relapse (UP ≥ 1.0 g/day)	2	6.9	9	47.4	0	0.0	11	20.4
Cr × 1.5 times	5[a]	17.2	0	0.0	0	0.0	5	9.3
Cr × 2 times	1	3.4	0	0.0	0	0.0	1	1.9
ESRD	0	0.0	0	0.0	0	0.0	0	0.0
Death	0	0.0	2[b]	10.5	0	0.0	2	3.7
Hospitalization due to infection	2	6.9	2	10.5	0	0.0	4	7.4
Use of anti-diabetic drugs	5	17.2	3	15.8	2	33.3	10	18.5
Malignancy	1	3.4	1	5.3	0	0.0	2	3.7
Immunosuppressive therapy	n	%	n	%	n	%	n	%
Oral prednisolone	25	86.2	18	94.7	6	100.0	49	90.7
IV methylprednisolone	1	3.4	2	10.5	3	50.0	6	11.1
Oral cyclophosphamide	2	6.9	0	0.0	0	0.0	2	3.7
Cyclosporine	17	58.6	3	15.8	3	50.0	23	42.6
Mizoribine	1	3.4	0	0.0	0	0.0	1	1.9
MMF	1	3.4	0	0.0	0	0.0	1	1.9

UP urinary protein, *Cr* creatinine, *ESRD* end-stage renal disease, *IV* intravenous, *MMF* mycophenolate mofetil

[a] One NS patient who was treated with supportive therapy involving renin–angiotensin system inhibitors (RAS-I), two patients with refractory NS (ICR-II) who were treated with PSL+CyA+RAS-I therapy, and two patients who achieved CR after PSL+CyA+RAS-I therapy

[b] One patient who died suddenly at 4 months after achieving CR, and another patient who died of infectious disease at the first relapse (at 6 months after achieving CR)

proliferative glomerulonephritis was treated with supportive therapy alone.

Clinical outcomes and complications of the elderly Japanese primary NS patients

All 19 (100 %) MCNS patients, including one patient who achieved spontaneous remission without immunosuppressive therapy, achieved CR. In addition, CR and ICR-I were achieved in 16 (55.2 %) and 18 (62.1 %) MN patients, 4 (66.7 %) and 5 (83.3 %) FSGS patients, 2 (66.7 %) and 3 (100 %) MPGN patients, and 1 (50 %) and 2 (100 %) CrGN patients, respectively. Relapse occurred in two (6.9 %) of the MN patients and nine (47.4 %) of the MCNS patients. As for renal dysfunction, it was observed in five (7.2 %) MN patients, including one patient who received supportive therapy with renin–angiotensin system inhibitors (RAS-I), two patients with refractory NS who were treated with PSL + CyA + RAS-I therapy, and two patients who achieved CR after PSL + CyA + RAS-I therapy (Table 4).

Among the three major pathological types of NS, serious complications were observed in eight (14.8 %) patients, i.e., two (3.7 %) patients died including one patient who died suddenly at 4 months after achieving CR and another patient who died of infectious disease at the first relapse, which occurred 6 months after achieving CR; four patients (7.4 %, two MCNS patients and two MN patients) who were hospitalized due to infectious disease; and two (3.7 %) patients who developed malignancies (Table 4). In addition, the initiation of anti-diabetic drug treatment was necessary in 14 of the 61 patients (23.0 %), and all 14 of these patients were treated with immunosuppressive drugs including PSL (25.0 %). In addition, the initial dosage of prednisolone was much higher in the group of the initiation of anti-diabetic drug treatment as compared with others (mean 39.3 ± 7.3 vs. 23.6 ± 14.3 mg/day, $p < 0.001$).

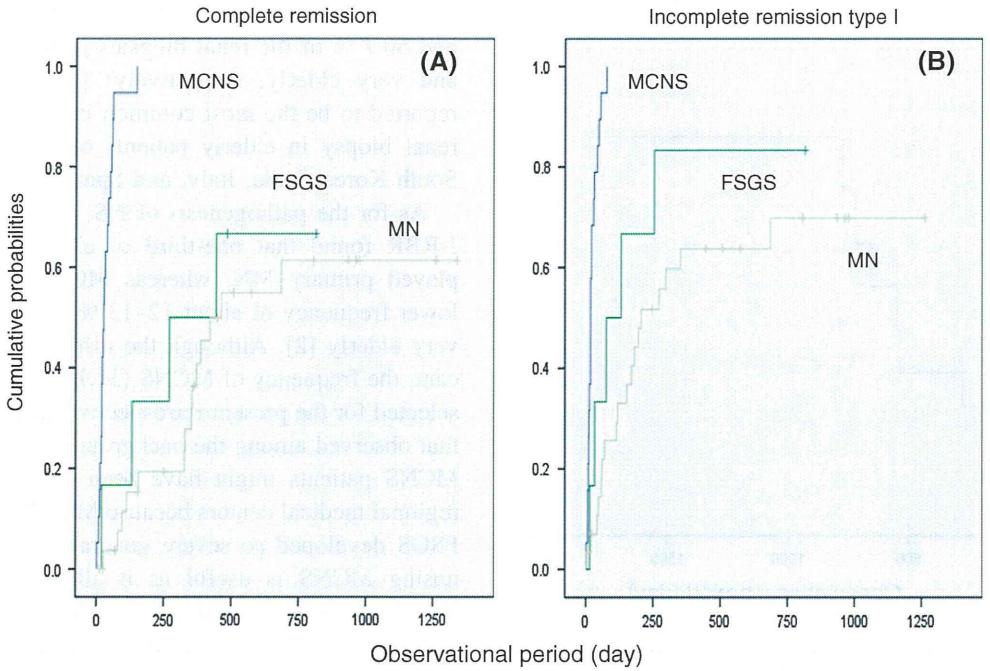

Fig. 1 Kaplan–Meier curves of remission among the elderly NS patients. There were significant differences in the median time to complete remission (a) or ICR-I (b) among the MCNS, FSGS, and MN patients (median: 26 days vs. 271 days vs. 461 days, respectively, log-rank test, Chi-square: 56.606, $p < 0.001$ for CR; 18 days vs. 76 days vs. 207 days, respectively, log-rank test, Chi-square: 44.655, $p < 0.001$ for ICR-I)

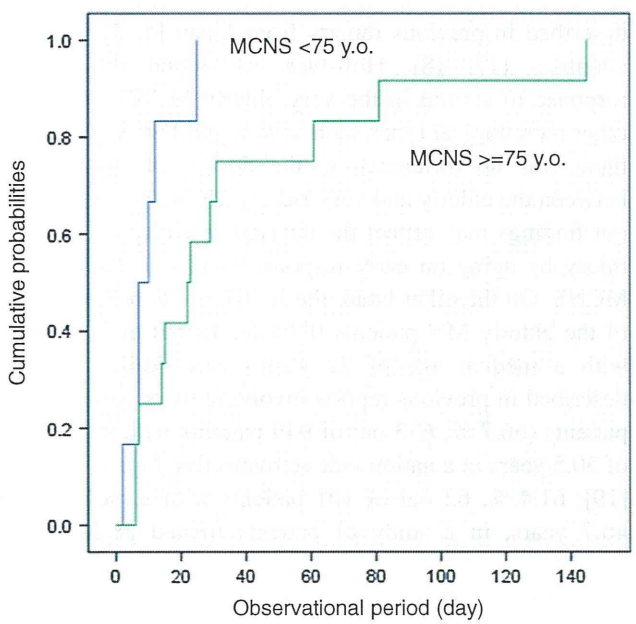

Fig. 2 Kaplan–Meier curves of complete remission among the elderly and very elderly MCNS patients. A significant difference in the median time to complete remission was detected between the elderly (65–74 years, $n = 7$) and very elderly (≥75 years old, $n = 12$) MCNS patients (median: 7 vs. 22 days, respectively, log-rank test, Chi-square: 4.333, $p = 0.037$)

Remission of primary NS in elderly and very elderly MN, MCNS, and FSGS patients

There was a significant difference in the median time to remission; i.e., CR (Kaplan–Meier analysis: 26 vs. 271 vs. 461 days, $p < 0.001$, Fig. 1a) or ICR-I (Kaplan–Meier analysis: 18 vs. 76 vs. 207 days, $p < 0.001$, Fig. 1b), among the patients with MCNS, FSGS, and MN.

In addition, the elderly (65–74 years, $n = 7$) MCNS patients exhibited a significantly shorter time to first remission than the very elderly (aged ≥ 75 years, $n = 12$) MCNS patients (median 7 vs. 22 days, $p = 0.037$, Fig. 2). However, there was no difference in the doses of prednisolone between elderly group (age <75 years, mean dose at 36.7 ± 9.6 mg/day) and very elderly group (age ≥75 years, mean dose at 37.0 ± 10.3 mg/day). After adjusting for clinically relevant factors such as gender, age, serum creatinine levels, serum albumin levels, initial prednisolone doses, and proteinuria using a Cox regression model, age (≥75 years) was identified as a significant predictor of later remission (Chi-square 4.094, $p = 0.043$). On the other hand, no significant difference in the time to first remission was detected between the elderly (17 MN and 2 FSGS patients) and very elderly patients in the MN or FSGS group (12 MN and 4 FSGS patients).

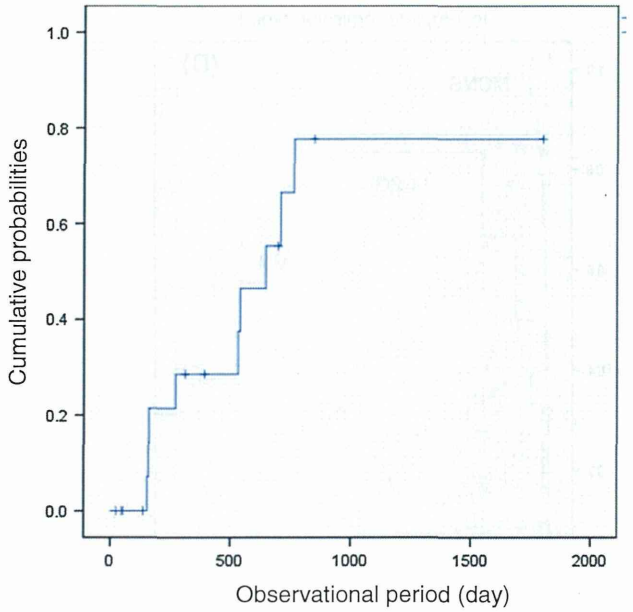

Fig. 3 Kaplan–Meier curves of the first relapse among the elderly MCNS patients. The estimated median time to relapse and the cumulative probability of a first relapse were 647 (95 % confidence interval, 466–828) days and 0.77, respectively

Age did not influence the relapse rate of the elderly or very elderly MCNS patients

Relapses occurred in nine (47.4 %) of the MCNS patients. However, there was no difference in the frequency of relapses between the elderly (65–74 years, $n = 3$, 50 %, one patient dropped out after achieving CR) and the very elderly MCNS patients (aged ≥75 years, $n = 6$, 50 %).

The median time to relapse was 402 days [interquartile range: 159–663]. According to Kaplan–Meier analysis, the estimated median time to relapse and the cumulative probability of a first relapse were 647 days (95 % confidence interval (CI), 466–828) and 0.77, respectively (Fig. 3).

Discussion and comments

The J-RBR represents the largest renal biopsy series of elderly (aged over 65 years) or very elderly (over 75 years) patients in the world [2]. While we cannot exclude the possibility that the J-RBR is subject to sampling bias, it is likely to be reasonably representative of the nationwide situation in Japan. In addition, it allows investigators to analyze the registered data in real time and to select patients with pathologically diagnosed renal conditions such as NS. Using the J-RBR, a previous study demonstrated that among elderly and very elderly Japanese renal biopsies are most commonly performed for NS (36.3 % and 50.7 % of the renal biopsies performed in the elderly and very elderly, respectively) [2]. Similarly, NS was reported to be the most common indication (37–64 %) for renal biopsy in elderly patients of over 60 years old in South Korea, India, Italy, and Spain [13–16].

As for the pathogenesis of NS, a previous study of the J-RBR found that one-third of elderly NS patients displayed primary MN, whereas MCNS exhibited a much lower frequency of about 12–13 % among the elderly and very elderly [2]. Although the difference was not significant, the frequency of MCNS (31.1 %) among the patients selected for the present retrospective study was higher than that observed among the background cohort (19.4 %). The MCNS patients might have been referred to appropriate regional medical centers because MCNS and some cases of FSGS developed so severe generalized edema, and diagnosing MCNS is useful as it allows the patient to be switched to steroid treatment, as shown in this study.

In this study, we detected a difference in the remission rates of elderly Japanese NS patients between the various pathological types of primary NS such as MCNS, FSGS, and MN. The response to immunosuppressive therapy was favorable (100 % of the MCNS patients achieved CR, and 83.3 % of the FSGS patients and 62.1 % of the MN patients achieved an ICR-I or CR) compared with those described in previous reports from Japan [4, 7] and other countries [17, 18]. However, we found the delayed response to steroid in the very elderly MCNS, but not in other pathological types such as MN and FSGS. Moreover, there was no difference in the dosage of prednisolone between the elderly and very elderly MCNS patients. Then, our findings may reflect the delayed recovery of podocyte injury by aging on early response to steroid as shown in MCNS. On the other hand, the ICR-I + CR remission rate of the elderly MN patients (62.1 %, 18 out of 29 patients with a median age of 72 years) was similar to those described in previous reports involving much younger MN patients (66.7 %, 633 out of 949 patients with a mean age of 50.5 years in a nationwide retrospective Japanese survey [19]; 61.4 %, 62 out of 101 patients with a mean age of 46.7 years, in a study of patients treated at Kanazawa University Hospital, Japan [20]). One of the interesting features of the present elderly MN cohort was the fact that 58.6 % of them were administered CyA. Kalliakmani et al. reported that administering low doses of CyA in combination with PSL resulted in the remission of NS in most patients with idiopathic MN [21]. As for FSGS, the ICR-I + CR remission rate observed in the present study (83.3 %, 5 out of 6 patients with a median age of 75 years) was better than that obtained in a nationwide retrospective Japanese survey performed in 2002 (51.8 %, 144 out of

278 patients with a mean age of 38.0 years) [22]. These findings have important implications for the assessment of immunosuppressive therapy in elderly Japanese NS patients. In future, an ongoing prospective study of NS (Japan Nephrotic Syndrome Cohort Study, J-NSCS) as mentioned in the annual report of J-RBR will provide more accurate data on the outcomes of NS patients in Japan [23].

As for the relapse rate of MCNS, the frequency and cumulative probability of a first relapse were 47.4 % and 0.77, respectively, among the elderly patients in the present study, and the median time to the first relapse was quite long (647 days, 95 %CI: 466–823 days). A recent Japanese report about a non-elderly MCNS patient cohort demonstrated that the relapse rates differed between the patients treated with oral PSL combined with intravenous mPSL therapy (mPSL + PSL, 46.2 %, 30 out of 65 patients; median time to relapse: 1-year [0.6–1.5]) and those administered oral PSL alone (66.7 %, 40 out of 60 patients; median time to relapse: 8 months [0.4–1.6]) [24]. Although intravenous mPSL therapy was only administered to two MCNS patients in the present study, their relapse rate was similar to that of the abovementioned mPSL+PSL group, which was derived from a much younger population. In addition, elderly MCNS patients displayed a much longer interval until the first relapse. Therefore, immunosuppressive therapy including corticosteroids might be effective for elderly Japanese MCNS patients.

On the contrary, renal dysfunction developed in five MN patients; i.e., one patient with prolonged NS who was treated with supportive therapy involving RAS-I, two patients with refractory NS who were treated with PSL+CyA+RAS-I therapy, and two patients who achieved CR after PSL+CyA+RAS-I therapy. These findings suggest that prolonged proteinuria, as was shown in previous studies [19, 20], and/or long-term combination treatment with RAS-I and CyA influence the risk of renal dysfunction, even after CR has been achieved. Similarly, it was reported that significant deterioration of histological lesions occurs with time even in MN patients who achieve remission, although MN patients do not display the typical features of CyA nephrotoxicity [21]. In this notion, CyA-related renal injury after remission in the elderly nephrotic syndrome was a future issue to answer by more large case studies.

In this study, two of the MCNS patients died, and two MCNS patients were hospitalized due to infectious disease. Thus, about 20 % of the elderly MCNS patients suffered serious complications. Infectious diseases are a life-threatening complication of NS. A previous report suggested that intravenous immunoglobulin injections might be useful for maintaining serum IgG levels of >600 mg/dL and for preventing infectious complications of NS [25]. In addition, the initiation of anti-diabetic drug treatment was necessary in 25.0 % of the 56 patients who were treated with steroids in this study. Diabetes is an important complication of NS in terms of its effects on the risk of infection as well as other disorders such as cardiovascular complications.

In future studies, these issues regarding the early diagnosis, treatment, and prevention of complications such as severe infectious diseases in elderly and very elderly NS patients should be resolved.

In conclusion, renal biopsy can provide valuable diagnostic information about elderly Japanese NS patients because the clinical outcomes and response to therapy differ between the various pathological types of NS. 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.

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Conflict of interest None of authors have any conflicts of interest to disclose for this paper.

Appendix

The following investigators and institutions have participated in the development of the J-RBR since 2007: Hirofumi Makino and Hitoshi Sugiyama (Okayama University), Takashi Taguchi (Nagasaki University; present institution: Nagasaki City Hospital), Hitoshi Yokoyama (Kanazawa Medical University), Hiroshi Sato (Tohoku University), Takao Saito and Yoshie Sasatomi (Fukuoka University), Yukimasa Kohda (Kumamoto University; present institution: Hikarinomori Clinic), Shinichi Nishi (Niigata University; present institution: Kobe University), Kazuhiko Tsuruya and Yutaka Kiyohara (Kyushu University), Hideyasu Kiyomoto (Kagawa University; present institution: Tohoku University), Hiroyuki Iida (Toyama Prefectural Central Hospital; present institution, Saiseikai Takaoka Hospital), Tamaki Sasaki (Kawasaki Medical School), Makoto Higuchi (Shinshu University), Motoshi Hattori (Tokyo Women's Medical University), Kazumasa

Oka (Osaka Kaisei Hospital; present institution: Hyogo Prefectural Nishinomiya Hospital), Shoji Kagami (The University of Tokushima Graduate School), Michio Nagata (University of Tsukuba), Tetsuya Kawamura (Jikei University School of Medicine), Masataka Honda (Tokyo Metropolitan Children's Medical Center), Yuichiro Fukasawa (KKR Sapporo Medical Center; present institution: Sapporo City Hospital), Atsushi Fukatsu (Kyoto University Graduate School of Medicine), Kunio Morozumi (Japanese Red Cross Nagoya Daini Hospital), Norishige Yoshikawa (Wakayama Medical University), Yukio Yuzawa (present institution: Fujita Health University), Seiichi Matsuo (Nagoya University Graduate School of Medicine) and Kensuke Joh (Chiba-East National Hospital; present institution: Sendai Shakai Hoken Hospital).

References

1. White Book of Aging from the Government of Japan. [http://www8.cao.go.jp/kourei/whitepaper/w-2011/gaiyou/pdf/1s1s.pdf] (Accessed, April 2, 2012)
2. Yokoyama H, Sugiyama H, Sato H, Taguchi T, Nagata M, Matsuo S, Makino H, Watanabe T, Saito T, Kiyohara Y, Nishi S, Iida H, Morozumi K, Fukatsu A, Sasaki T, Tsuruya K, Kohda Y, Higuchi M, Kiyomoto H, Goto S, Hattori M, Hataya H, et al. Renal disease in the elderly and the very elderly Japanese: analysis of the Japan Renal Biopsy Registry (J-RBR). Clin Exp Nephrol. 2012;16:903–20.
3. Sato H, Saito T, Furuyama T, Yoshinaga K. Histologic studies on the nephrotic syndrome in the elderly. Tokoku J Exp Med. 1987;53:259–64.
4. Komatsuda A, Nakamoto Y, Imai H, Yasuda T, Yanagisawa MM, Wakui H, Ishino T, Satoh K, Miura AB. Kidney diseases among the elderly- A clinicopathological analysis of 247 elderly patients. Intern Med. 1993;32:377–81.
5. Ozono Y, Harada T, Yamaguchi K, Tamura K, Hara K, Taguchi T. Nephrotic syndrome in the elderly-clinicopathological study. Nihon Jinzo Gakkai Shi. 1994;36:44–50.
6. Uezono S, Hara S, Sato Y, Komatsu H, Ikeda N, Shimao Y, Hayashi T, Asada Y, Fujimoto S, Eto T. Renal biopsy in elderly patients: a clinicopathological analysis. Ren Fail. 2006;28:549–55.
7. Omokawa A, Komatsuda A, Nara M, Fujiwara T, Sato R, Togashi M, Okuyama S, Sawada K, Wakui H. Renal biopsy in patients aged 80 years and older: a single-center experience in Japan. Clin Nephrol. 2012;77:461–7.
8. Verde E, Quiroga B, Rivera F, Lopez-Gomez JM. Renal biopsy in very elderly patients: Data from the Spanish Registry of Glomerulonephritis. Am J Nephrol. 2011;35:230–7.
9. Tse KC, Lam MF, Yip PS, Li FK. Idiopathic minimal change nephrotic syndrome in older adults: steroid responsiveness and pattern of relapses. Nephrol Dial Transplant. 2003;18:1316–20.
10. Sugiyama H, Yokoyama H, Sato H, Saito T, Kohda Y, Nishi S, et al. Committee for Standardization of renal pathological diagnosis and working group for renal biopsy database, Japanese society of nephrology, Tokyo, Japan: Japan renal biopsy registry: Japan renal biopsy registry: the first nationwide, web-based, and prospective registry system of renal biopsies in Japan. Clin Exp Nephrol. 2011;15:493–503.
11. Churg J, Bernstein J, Glassock RJ, editors. Renal disease: classification and atlas of glomerular diseases. 2nd ed. New York: Igaku-shoin; 1995. p. 4–20.
12. Matsuo S, Imai E, Saito T, Taguchi T, Yokoyama H, Narita I. Guidelines for the treatment of nephrotic syndrome. Jpn J Nephrol. 2011;53:136–41.
13. Shin JH, Pyo HJ, Kwon YJ, Chang MK, Kim HK, Won NH, Lee HS, Oh KH, Ahn C, Kim S, Lee JS. Renal biopsy in elderly patients: clinicopathological correlation in 117 Korean patients. Clin Nephrol. 2001;56:19–26.
14. Prakash J. Singh AK, Saxena RK, Usha. Glomerular diseases in the elderly in India. Int Urol Nephrol. 2003;35:283–8.
15. Ferro G, Dattolo P, Nigrelli S, Michelassi S, Pizzarelli F. Clinical pathological correlates of renal biopsy in elderly patients. Clin Nephrol. 2006;65:243–7.
16. Rivera F. Lopez-Gomez JM, Perez-Garcia R: Clinicopathologic correlations of renal pathology in Spain. Kidney Int. 2004;66:898–904.
17. Cameron JS. Nephrotic syndrome in the elderly. Semin Nephrol. 1996;16:319–29.
18. Yoon HY, Shin MJ, Kim YS, Choi BS, Kim BS, Choi YJ, Kim YO, Yoon SA, Kim YS, Yang CW. Clinical impact of renal biopsy on outcomes in elderly patients with nephrotic syndrome. Nephron Clin Pract. 2011;117:c20–7.
19. Shiiki H, Saito T, Nishitani Y, Mitarai T, Yorioka N, Yoshimura A, Yokoyama H, Nishi S, Tomino Y, Kurokawa K, Sakai H. Prognosis and risk factors for idiopathic membranous nephropathy with nephrotic syndrome in Japan. Kidney Int. 2004;65:1400–7.
20. Yoshimoto K, Yokoyama H, Wada T, Furuichi K, Sakai N, Iwata Y, Goshima S, Kida H, Kobayashi K. Pathologic findings of initial biopsies reflect the outcomes of membranous nephropathy. Kidney Int. 2004;65:148–53.
21. Kalliakmani P, Koutroulia E, Sotsiou F, Vlachojannis JG, Goumenos DS. Benefit and cost from the long-term use of cyclosporine—a in idiopathic membranous nephropathy. Nephrology (Carlton). 2010;15:762–7.
22. Sakai H, Kurokawa K, Saito T, Shiiki H, Nishi S, Mitarai T, Yokoyama H, Yoshimura A, Yorioka T. Guidelines for the treatment of refractory nephrotic syndrome in adult. Jpn J Nephrol. 2002;44:751–61.
23. Sugiyama H, Yokoyama H, Sato H, Saito T, Kohda Y, Nishi S, Tsuruya K, Kiyomoto H, Iida H, Sasaki T, Higuchi M, Hattori M, Oka K, Kagami S, Kawamura T, Takeda T, Hataya H, Fukasawa Y, Fukatsu A, Morozumi K, Yoshikawa N, Shimizu A, Kitamura H, Yuzawa Y, Matsuo S, Kiyohara Y, Joh K, Nagata M, Taguchi T, Makino H. Committee for standardization of renal pathological diagnosis; committee for kidney disease registry; Japanese society of nephrology. Japan renal biopsy registry and Japan kidney disease registry: committee report for 2009–2010. Clin Exp Nephrol. 2013;17:155–73.
24. Shinzawa M. Ryohei Yamamoto R, Nagasawa Y, Oseto S, Mori D, Tomida K, Hayashi T, Izumi M, Fukunaga M, Yamauchi A, Tsubakihara Y, Isaka Y. Comparison of methylprednisolone plus prednisolone with prednisolone alone as initial treatment in adult-onset minimal change disease: a retrospective cohort study. Clin J Am Soc Nephrol. 2014;9:1040–8.
25. Ogi M, Yokoyama H, Tomosugi N, Hisada Y, Ohta S, Takaeda M, Wada T, Naito T, Ikeda K, Goshima S, Takasawa K, Kobayashi K. Risk factors for infection and immunoglobulin replacement therapy in adult nephrotic syndrome. Am J Kidney Dis. 1994;24:427–36.

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ORIGINAL ARTICLE

Clinical significance of serum and urinary soluble urokinase receptor (suPAR) in primary nephrotic syndrome and MPO-ANCA-associated glomerulonephritis in Japanese

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Abstract
Background The soluble urokinase receptor (suPAR) has been implicated as a cause of primary focal segmental glomerulosclerosis (FSGS). However, the clinical significance of suPAR in glomerular diseases currently remains unclear.
Methods In this retrospective single-center cohort study, we investigated serum (s-) and urinary (u-) suPAR in patients with primary nephrotic syndrome (NS) (serum/urine: 37/32 cases) and MPO-ANCA-associated glomerulonephritis (ANCA-GN) (serum/urine: 13/11 cases).
Results In pretreatment s- and u-suPAR, no significant differences were observed between the primary NS and ANCA-GN groups or among the pathological types of primary NS. An inverse correlation was noted between pretreatment s-suPAR and eGFR in the primary NS and ANCA-GN groups. A positive correlation was noted between pretreatment u-suPAR and proteinuria in the primary NS group. Furthermore, time-course changes in s- and u-suPAR over 2 months after therapy were associated with the therapeutic responsiveness of primary NS, particularly the differentiation of MCNS from FSGS (s-suPAR: AUC-ROC = 0.905, $p = 0.007$; u-suPAR: AUC-ROC = 0.816, $p = 0.048$). In the ANCA-GN group, a positive correlation was found between pretreatment s-suPAR and clinical severity or crescent formation, whereas u-suPAR was not correlated with these parameters.
Conclusion S- and u-suPAR after therapy may serve as clinical markers to judge the treatment response of untreated NS and differentiate MCNS from FSGS, but not in pretreatment patients. S-, but not u-suPAR may predict the severity of and crescent formation in ANCA-GN.

Keywords suPAR · Nephrotic syndrome · MPO-ANCA-associated glomerulonephritis

Introduction

Focal segmental glomerulosclerosis (FSGS) is a typical disease that manifests steroid-resistant, intractable nephrotic syndrome (NS). Reiser's group reported that the serum-soluble urokinase receptor (s-suPAR) bound to and activated β3 integrin on glomerular podocytes and induced proteinuria and FSGS-like lesions [1–3]. They concluded that suPAR was a humoral factor involved in the development of FSGS. In their study, s-suPAR was high in 2/3 of primary and recurrent FSGS patients after kidney transplantation, whereas no elevation in s-suPAR was noted in patients with other glomerular diseases. FSGS-like lesions can be prevented by the removal of circulating s-suPAR with an anti-suPAR antibody treatment, plasmapheresis, a β3 integrin-inhibitory, low-molecular-weight substance, and cycloRGDfv. Huang et al. [4] reported that s-suPAR was significantly higher in patients with crescentic FSGS than in those with non-crescentic FSGS. They also recently demonstrated that urinary suPAR (u-suPAR) was specifically elevated in patients with primary FSGS and was associated with disease severity [5].

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Recent studies did not discriminate between FSGS, minimal change diseases, or steroid-responsive illnesses in pediatric patients with NS and in patients with biopsy-proven idiopathic FSGS or with non-FSGS diseases [6, 7]. Wada et al. [8] demonstrated that s-suPAR was not a useful clinical marker for FSGS patients in Japan. Otherwise, time-course changes of s-suPAR after therapy could not be assessed in this paper, because of a multicenter cross-sectional study. Moreover, they did not study u-suPAR in Japanese.

The aim of our longitudinal study was to clarify the predictability of long-term therapeutic responses by assessing time-course changes in s- and u- suPAR after therapy. We also investigated the usefulness of suPAR in differentiating FSGS from MCNS, and the clinical significance of suPAR in a typical crescentic disease, MPO-ANCA-associated glomerulonephritis (ANCA-GN).

Materials and methods

Patient population

S-suPAR was measured before the administration of treatments in 37 patients diagnosed with primary NS and treated at Kanazawa Medical University (8 FSGS, 12 MCNS, 15 MN, and 2 MPGN patients) and 13 ANCA-GN patients. U-suPAR was also measured in 32 patients with primary NS (8 FSGS, 10 MCNS, 12 MN, and 2 MPGN patients) and 11 ANCA-GN patients. eGFR was calculated using the predictive equation for Japanese people [9], and urinary protein excretion was determined from the urinary protein/Cr ratio. Renal biopsy was performed in all NS patients and 10 of the ANCA-GN patients. S-suPAR after the initiation of immunosuppressive therapy was measurable in 7 FSGS, 9 MCNS, 9 MN, and 2 MPGN patients. U-suPAR after therapy was also measurable in 7 FSGS, 7 MCNS, 6 MN, and 2 MPGN patients. The time-course changes induced by immunosuppressive therapy were investigated in these patients. S- and u-suPAR were also measured in 20 healthy volunteers. The protocol of this study was approved by the Clinical Study Ethics Review Board of Kanazawa Medical University (No. 197). Prior to this study, verbal/written informed consent was obtained from all patients. The present study was conducted according to principles of the Declaration of Helsinki.

Immunosuppressive therapy protocol

Of all primary NS patients, 71 % (including all FSGS and MCNS patients) received the protocol immunosuppressive therapy: methylprednisolone pulse (MPT)/prednisolone (PSL)/cyclosporine (CyA) combination therapy (Table 1), in which MP was administered for 3 days at 500 mg/day, followed by oral PSL and CyA. PSL was initially administered at 20 mg, while CyA was given at 2 mg/kg/day. These doses were adjusted to target CyA levels of 600–800 ng/mL by 2 h after administration.

Definition of the treatment response of NS and clinical severity of ANCA-GN

The responses of NS to the treatment were assessed based on the definition of the Japanese Society of Nephrology [10]: urinary protein less than 0.3 g/day, between 0.3 and below 1.0 g/day, between 1.0 and below 3.5 g/day, and 3.5 g/day or greater were defined as complete remission (CR), incomplete remission (ICR)-I and -II, and ineffective, respectively. When urinary protein was not decreased to below 1 g/day by the various treatments including steroids and immunosuppressors for 6 months, the condition of the patient was defined as intractable NS.

The clinical severity of ANCA-GN was determined based on the definition of the Japanese Society of Nephrology [11].

Measurement of serum and urinary suPAR

suPAR was measured in sera and urine frozen at −80 °C using a commercial ELISA kit, the Quantikine Human suPAR Immunoassay (R&D Systems, Minneapolis, MN, USA) following the manufacturer's protocol.

Statistical analysis

Data of continuous variables are presented as medians with interquartile ranges. Dunn's test (nonparametric) was used for multiple comparisons of pretreatment s-/u-suPAR and clinical parameters. To compare UP before the treatment between the primary NS and ANCA-GN groups, the Mann–Whitney U test was used. Spearman's correlation coefficient test was used to evaluate the relationships between pretreatment s-/u-suPAR and clinical parameters. However, statistical analysis could not be performed about the correlation between pretreatment u-suPAR and CRP, because almost cases showed negative CRP levels. To compare s- and u-suPAR levels (eGFR, CRP, UP) in the primary NS group between before and after 2 months of treatment, the Wilcoxon's signed-rank test was used. Multiple regression analyses were performed to evaluate the relationship between therapeutic responses (i.e., intractable NS or non-intractable NS) and changes in s-suPAR during 2 months after therapy or s-suPAR at 2 months after therapy while controlling for eGFR and CRP. A ROC analysis was used to evaluate the accuracy of differentiation based on s- and u-suPAR. Stat Flex Ver6

Table 1 Demographic/clinical characteristics

	Primary nephrotic syndrome					ANCA-GN	Normal control
	Total *n* = 37	FSGS *n* = 8	MCNS *n* = 12	MN *n* = 15	MPGN *n* = 2	*n* = 13	*n* = 20
Urinary suPAR measurable *n* (%)	32 (86.5)	8 (100.0)	10 (83.3)	12 (80.0)	2 (100.0)	11 (84.6)	20 (100.0)
Baseline characteristics							
Age (years)	60.0 (40.0–68.0)	48.0 (29.0–68.0)	47.0 (33.5–61.0)[a]	66.0 (60.8–71.3)	24, 76	69.0 (62.3–77.0)	29.5 (25.5–34.0)[A, B]
Gender (male %)	59.5	50.0	58.3	73.3	100.0	46.2	75.0
UP (g/gCr)	9.3 (7.2–12.4)[C]	11.5 (9.7–15.9)	11.4 (9.4–14.3)	7.4 (3.5–8.5)[b, c]	5.6, 9.3	1.2 (0.6–2.0)	Not done*
Selectivity index	0.20 (0.13–0.25)	0.19 (0.16–0.22)	0.14 (0.09–0.24)	0.21 (0.20–0.31)	0.18, 0.38	Not done	Not done
eGFR (mL/min/1.73 m^2)	62.5 (30.4–78.6)[D, E]	39.5 (25.9–79.1)	68.2 (50.2–78.2)	62.5 (28.5–77.1)	95.5, 52.1	14.1 (7.0–39.7)	90.7 (82.1–97.8)
sAlb (g/dL)	1.90 (1.30–2.20)	2.10 (1.55–2.15)	1.30 (1.15–1.40)[d]	2.10 (1.83–2.50)	2.60, 1.80	3.10 (2.20–3.25)[F, G]	5.10 (5.00–5.20)
TC (mg/dL)	343.0 (272.0–437.0)[H, I]	362.5 (325.5–417.5)	469.0 (349.0–514.5)[e]	269.0 (257.0–373.0)	285.0, 233.0	167.0 (149.0–213.3)	202.0 (168.0–225.5)
CRP (mg/dL)	0.10 (0.10–0.26)[J, K]	0.10 (0.10–0.22)	0.22 (0.10–0.34)	0.10 (0.10–0.10)	0.10, 0.10	3.55 (0.34–8.33)	0.05 (0.02–0.08)
Treatments *n* (%)							
MPT + PSL + CyA	25 (67.6)	8 (100.0)	11 (91.7)	5 (33.3)	1 (50.0)	0 (0)	
MPT + PSL + MZB	2 (5.4)	0 (0)	0 (0)	1 (6.7)	1 (50.0)	1 (7.7)	
PSL + CyA	1 (2.7)	0 (0)	0 (0)	1 (6.7)	0 (0)	0 (0)	
PSL + MZB	2 (5.4)	0 (0)	0 (0)	2 (13.3)	0 (0)	0 (0)	
PSL alone	1 (2.7)	0 (0)	0 (0)	1 (6.7)	0 (0)	1 (7.7)	
MPT + PSL + IVCY	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (15.4)	
MPT + PSL	1 (2.7)	0 (0)	1 (8.3)	0 (0)	0 (0)	6 (46.1)	
MPT + PSL + POCY	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (15.4)	
PSL + IVCY	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (7.7)	
Others	5 (13.5)	0 (0)	0 (0)	5 (33.3)	0 (0)	0 (0)	
Outcomes at 6 months							
Urinary protein *n* (%)							
CR	24 (64.9)	7 (87.5)	12 (100.0)	4 (26.7)	1 (50.0)	–	
ICR-I	3 (8.1)	0 (0)	0 (0)	3 (20.0)	0 (0)	–	
ICR-II	8 (21.6)	1 (12.5)	0 (0)	6 (40.0)	1 (50.0)	–	
NS	2 (5.4)	0 (0)	0 (0)	2 (13.3)	0 (0)	–	
Renal function *n* (%)							
ESRD(permanent HD)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (23.1)	
Temporary HD	1 (2.7)	0 (0)	1 (8.3)	0 (0)	0 (0)	2 (15.4)	