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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

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, $p < 0.001$).

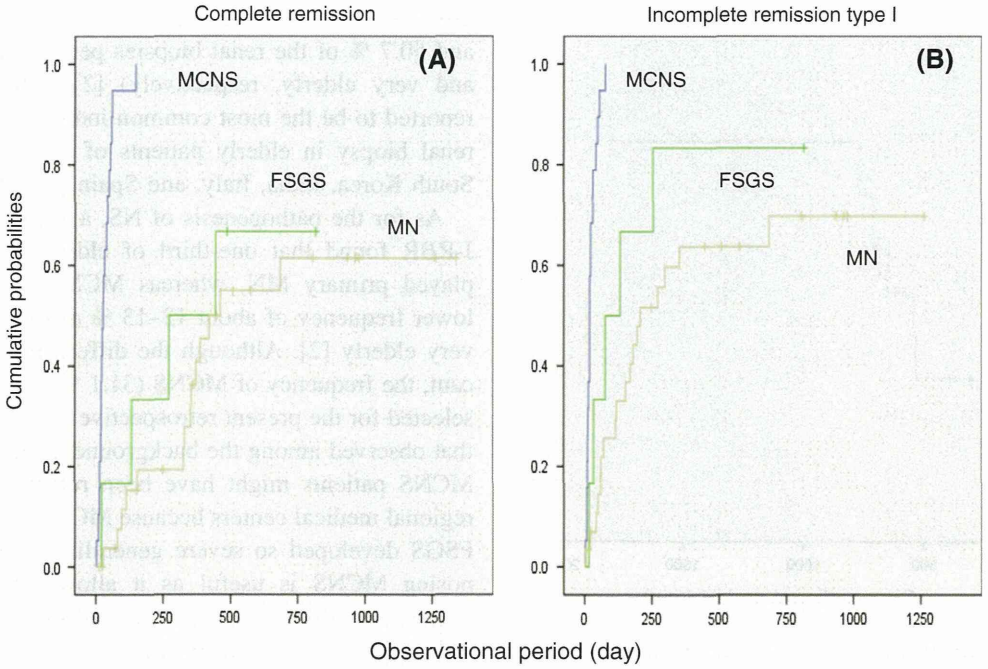

Fig. 1 Kaplan–Meier curves of remission among the elderly NS patients. There were significant differences in the median time to complete remission (a) or ICR-I (b) among the MCNS, FSGS, and MN patients (median: 26 days vs. 271 days vs. 461 days, respectively, log-rank test, Chi-square: 56.606, $p < 0.001$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

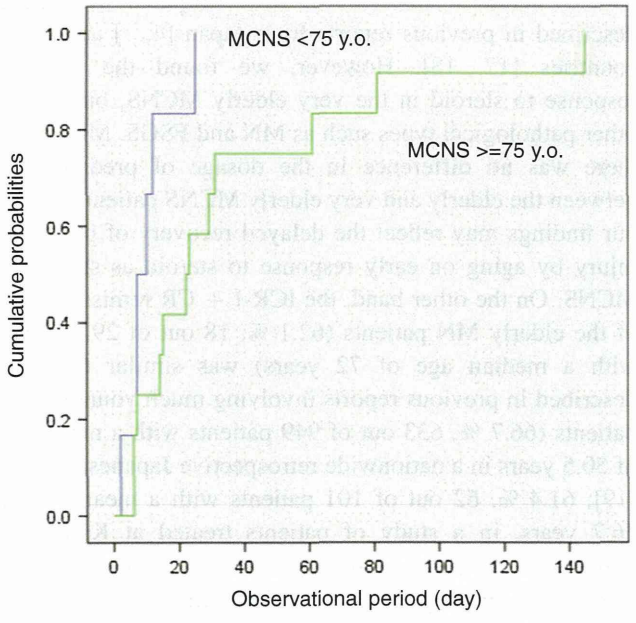

Fig. 2 Kaplan–Meier curves of complete remission among the elderly and very elderly MCNS patients. A significant difference in the median time to complete remission was detected between the elderly (65–74 years, $n = 7$) and very elderly (≥75 years old, $n = 12$) MCNS patients (median: 7 vs. 22 days,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

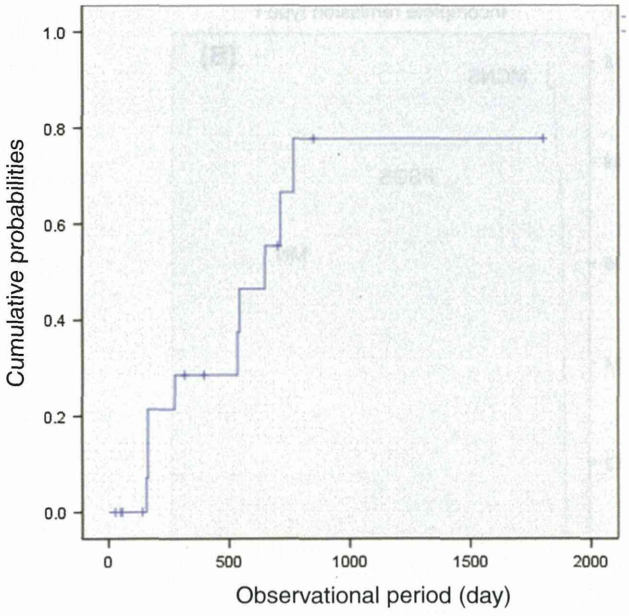

Fig. 3 Kaplan–Meier curves of the first relapse among the elderly MCNS patients. The estimated median time to relapse and the cumulative probability of a first relapse were 647 (95 % confidence interval, 466–828) days and 0.77, respectively

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).

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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)	

Table 1 continued

	Primary nephrotic syndrome					ANCA-GN	Normal control
	Total $n = 37$	FSGS $n = 8$	MCNS $n = 12$	MN $n = 15$	MPGN $n = 2$	$n = 13$	$n = 20$
Not required HD	36 (97.3)	8 (100.0)	11 (91.7)	15 (100.0)	2 (100.0)	8 (61.5)	

The data are presented as median (interquartile range). The multiple comparisons for clinical parameters among primary NS (total), ANCA-GN and control or among the pathological types of primary NS (FSGS vs. MCNS vs. MN) were performed by nonparametric test (Dunn test). The comparison for UP between primary NS and ANCA-GN was performed by Mann–Whitney *U* test

FSGS focal segmental glomerulosclerosis, *MCNS* minimal change nephrotic syndrome, *MN* membranous nephropathy, *MPGN* membranoproliferative glomerulonephritis, *ANCA-GN* anti-neutrophil cytoplasmic antibody positive glomerulonephritis, *UP* indicates urinary protein, *Alb* albumin, *TC* total cholesterol, *CRP* C-reactive protein, *MPT* methylprednisolone pulse therapy, *PSL* prednisolone, *CyA* cyclosporine A, *Mz* mizoribine, *CY* cyclophosphamide, *IV* intravenous, *PO* per os, *CR* complete remission, *ICR* incomplete remission, *NS* nephrotic syndrome, *ESRD* end-stage renal disease, *HD* hemodialysis

[A] $p < 0.01$ vs. primary NS (total), [B] $p < 0.01$ vs. ANCA-GN, [C] $p = 0.000001$ vs. ANCA-GN, [D] $p < 0.01$ vs. ANCA-GN, [E] $p < 0.01$ vs. Control, [F] $p < 0.05$ vs. primary NS (total), [G] $p < 0.01$ vs. control, [H] $p < 0.01$ vs. ANCA-GN, [I] $p < 0.01$ vs. control, [J] $p < 0.01$ vs. ANCA-GN, [K] $p < 0.01$ vs. control, [a] $p < 0.05$ vs. MN, [b] $p < 0.05$ vs. FSGS, [c] $p < 0.05$ vs. MCNS, [d] $p < 0.01$ vs. MN, [e] $p < 0.01$ vs. MN

* The urinary proteins of all normal controls were negative in qualitative analysis

(Artech Co., Ltd., Osaka, Japan) was used as the statistical analysis software. A p value of less than 0.05 was regarded as significant.

Results

Clinical characteristics of primary NS and ANCA-GN patients

The clinical characteristics of primary NS and ANCA-GN patients before therapy are shown in Table 1. Primary NS was diagnosed by renal biopsy in all patients. Of the ANCA-GN patients, 10 of those examined by renal biopsy were histologically diagnosed with crescentic glomerulonephritis. eGFR was significantly lower and CRP was higher in the ANCA-GN group than in the primary NS group (eGFR, $p < 0.01$; CRP, $p < 0.01$). Urinary protein (UP) was significantly lower in the ANCA-GN group than in the primary NS group ($p < 0.00001$). No significant differences were observed in eGFR, CRP, or the selectivity index among the primary NS disease types.

Comparison of pretreatment serum and urinary suPAR

S-suPAR before immunosuppressive therapy was significantly higher in the primary NS and ANCA-GN groups than in the control group ($p < 0.01$, Fig. 1a). On the other hand, no significant differences were noted in s-suPAR before immunosuppressive therapy between the primary NS and ANCA-GN groups (Fig. 1a) or among the disease types of primary NS (Fig. 1b).

U-suPAR before immunosuppressive therapy was also significantly higher in the primary NS and ANCA-GN groups than in the control group ($p < 0.01$, Fig. 1c). Similar to the serum results, no significant differences were noted in u-suPAR before immunosuppressive therapy between the primary NS and ANCA-GN groups (Fig. 1c) or among the disease types of primary NS (Fig. 1d).

Relationships between pretreatment serum and urinary suPAR and clinical parameters

Regarding relationships between pretreatment s-suPAR and clinical parameters (primary NS, Fig. 2; ANCA-GN, Fig. 3), a significant inverse correlation with eGFR was noted in the primary NS and ANCA-GN groups (primary NS, $n = 37$, $\rho = -0.677$, $p < 0.001$, Fig. 2b; ANCA-GN, $n = 13$, $\rho = -0.676$, $p = 0.011$, Fig. 3b). A significant positive correlation with CRP was also noted in the ANCA-GN group ($n = 13$, $\rho = 0.702$, $p = 0.008$, Fig. 3c). A significant positive correlation was noted in the primary NS group ($n = 32$, $\rho = 0.576$, $p = 0.001$,

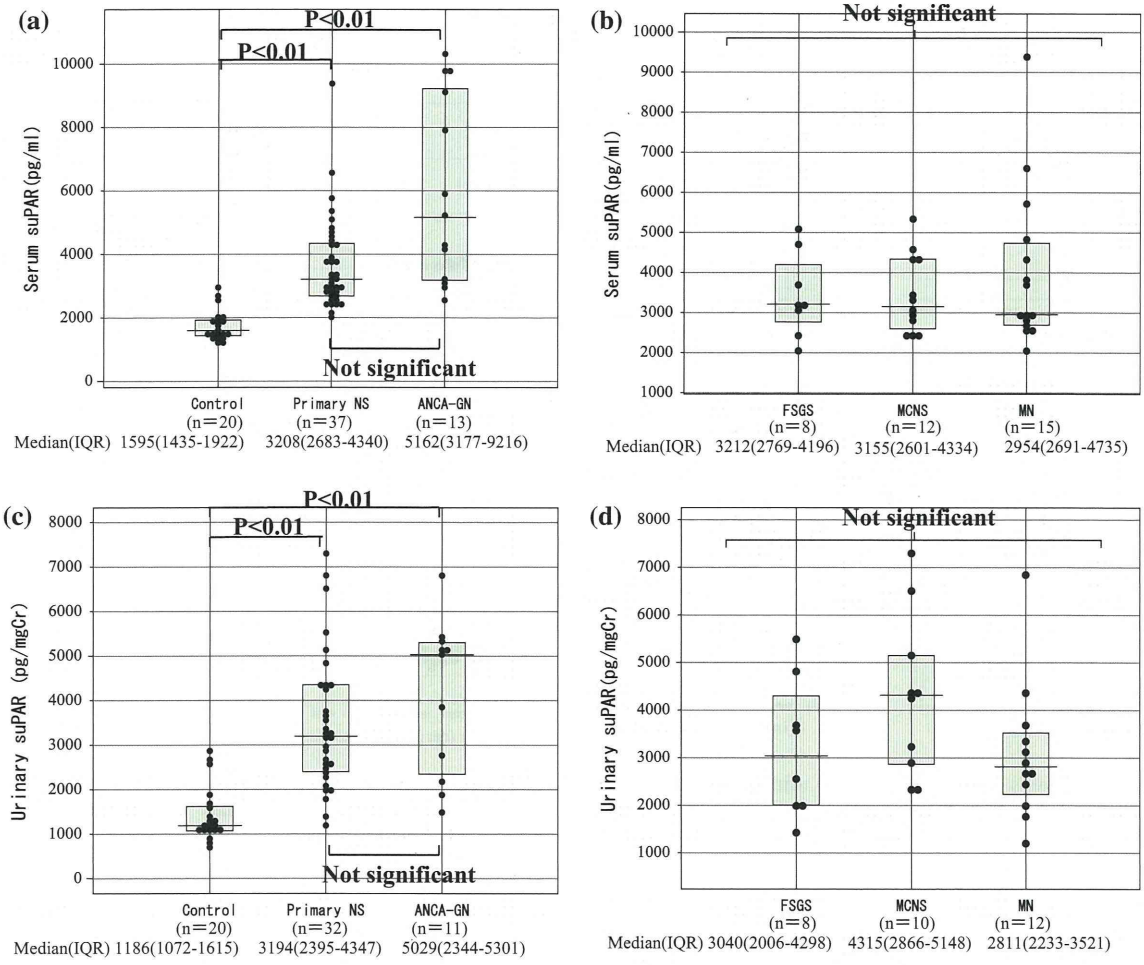

Fig. 1 Comparison of serum and urinary suPAR levels before therapy **a** Serum suPAR levels before therapy were significantly higher in patients with primary NS or ANCA-GN than in normal controls ($p < 0.01$). On the other hand, no significant differences were noted in pretreatment serum suPAR levels between primary NS and ANCA-GN. **b** There were no significantly differences among patients with FSGS, MCNS, MN in serum suPAR levels before therapy. **c** Urinary suPAR levels before therapy were significantly higher in patients with Primary NS or ANCA-GN than in normal controls ($p < 0.01$). On the other hand, no significant differences were noted in pretreatment urinary suPAR levels between primary NS and ANCA-GN. **d** There were no significant differences among patients with FSGS, MCNS, MN in urinary suPAR levels before therapy

supplement figure 1a) between s- and u-suPAR, but not in the ANCA-GN group ($n = 11$, $\rho = 0.245$, $p = 0.467$, supplement figure 1b).

Regarding the relationships between pretreatment u-suPAR and clinical parameters (primary NS, Fig. 2; ANCA-GN, Fig. 3), a significant positive correlation with CRP was noted in the ANCA-GN group ($n = 11$, $\rho = 0.656$, $p = 0.028$, Fig. 3f). U-suPAR positively correlated with UP in the primary NS group ($n = 32$, $\rho = 0.501$, $p = 0.003$, Fig. 2e), but inversely correlated with UP in the ANCA-GN group ($n = 11$, $\rho = -0.864$, $p = 0.001$, Fig. 3d).

Alterations in UP, serum suPAR, and urinary suPAR after immunosuppressive therapy

S-suPAR was measured for 2 months after the initiation of immunosuppressive therapy in 7 FSGS, 9 MCNS, 9 MN, and 2 MPGN patients. The relationship between changes in s-suPAR over 2 months of immunosuppressive therapy (Δ2M s-suPAR) and the treatment response of NS was investigated in these patients. Four MN, 1 FSGS (NOS), and 1 MPGN patients were intractable for therapy. S-suPAR during the 2-month period significantly decreased in MCNS ($n = 9$, 2996 [IQR 2677–4315] pg/mL before treatment vs. 2601 [IQR 2392–2882] pg/mL at 2 months of treatment, $p = 0.011$, Fig. 4a), and in non-intractable NS ($n = 21$, 2996 [IQR 2664–3739] vs. 2638 [IQR 2453–3242] pg/mL, $p = 0.023$). In contrast, s-suPAR during the 2-month period significantly increased in intractable NS ($n = 6$, 4249 [IQR 2939–6580] vs. 5267 [IQR 3474–8243] pg/mL, $p = 0.046$). No significant changes were noted in s-suPAR throughout the 2-month period in FSGS or MN (FSGS, $n = 7$, 3208 [IQR 2610–3605] vs. 2840 [IQR 2549–4668] pg/mL, $p = 0.499$,

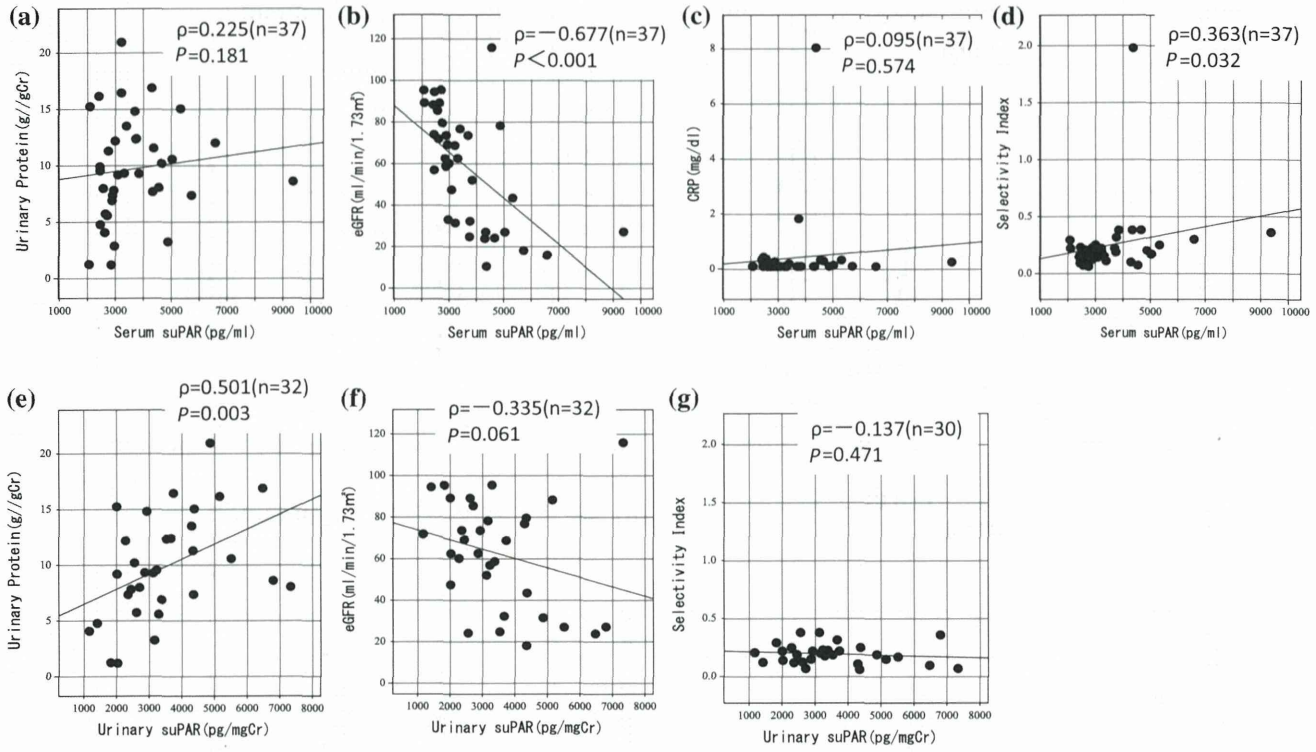

Fig. 2 The correlations between serum or urinary suPAR levels before therapy and the clinical parameters in primary NS. **a** Serum suPAR and urinary protein, **b** serum suPAR and eGFR, **c** serum suPAR and CRP, **d** serum suPAR and selectivity index, **e** urinary suPAR and urinary protein, **f** urinary suPAR and eGFR, **g** urinary suPAR and selectivity index

Fig. 4b; MN, $n = 9$, 2954 [IQR 2810–4545] vs. 3898 [IQR 2779–5227] pg/mL, $p = 0.110$, Fig. 4c). However, there were no significant changes in eGFR and CRP in these cases (data not shown). On the other hand, UP was significantly decreased in FSGS, MCNS, MN (supplement figure 2), non-intractable NS ($n = 21$, 11.30 [IQR 7.24–15.10] vs. 0.04 [IQR 0.01–0.14] g/gCr, $p < 0.001$), and intractable NS ($n = 6$, 8.95 [7.82–10.20] vs. 2.61 [1.91–3.94] g/gCr, $p = 0.028$) after therapy.

In addition, u-suPAR was measured for 2 months after the initiation of immunosuppressive therapy in 7 FSGS, 7 MCNS, 6 MN, and 2 MPGN patients. The relationship between changes in u-suPAR over 2 months of immunosuppressive therapy (Δ2M u-suPAR) and the treatment response of NS was investigated in these patients. Three MN, 1 FSGS (NOS), and 1 MPGN patient were intractable for therapy. U-suPAR significantly decreased during the 2-month period in MCNS ($n = 7$, 4341 [IQR 2478–4953] vs. 2148 [IQR 1568–2882] pg/mgCr, $p = 0.018$, Fig. 4d) or in non-intractable NS ($n = 17$, 3379 [IQR 2331–4348] vs. 2552 [IQR 1834–3148] pg/mgCr, $p = 0.039$). However, no significant changes were noted in u-suPAR throughout the 2-month period in FSGS or MN (FSGS, $n = 7$, 2550 [IQR 2001–3682] vs. 2925 [IQR 1969–4318] pg/mgCr, $p = 0.735$, Fig. 4e; MN, $n = 6$, 3041 [IQR 2441–3664] vs. 3023 [IQR 2552–3774] pg/mgCr, $p = 0.345$, Fig. 4f).

Significant factors affecting Δ2M s-suPAR and 2M suPAR

When we examined the factors affecting Δ2M s-suPAR using multiple regression analysis, the therapeutic response was identified as a significant factor, whereas CRP and eGFR were not (data shown in supplement Table 1). Moreover, a significant positive correlation was observed between s-suPAR at 2 months of treatment (2M s-suPAR) and UP at 2 months of treatment ($n = 27$, $\rho = 0.518$, $p = 0.006$). The multiple regression analysis suggested that the factors affecting 2M s-suPAR were the therapeutic response and eGFR, with the therapeutic response being the most influential factor (data shown in supplement Table 2).

What is the useful parameter for defining the new standard of therapeutic response at the early phase after therapy in Japanese patients with NS?

ROC analyses were performed to define the new standard of therapeutic response of NS at the early phase after therapy which was consistent with the conventional Japanese definition of therapeutic response at 6 months after therapy. The patients with primary NS could be divided into intractable NS group and non-intractable NS group

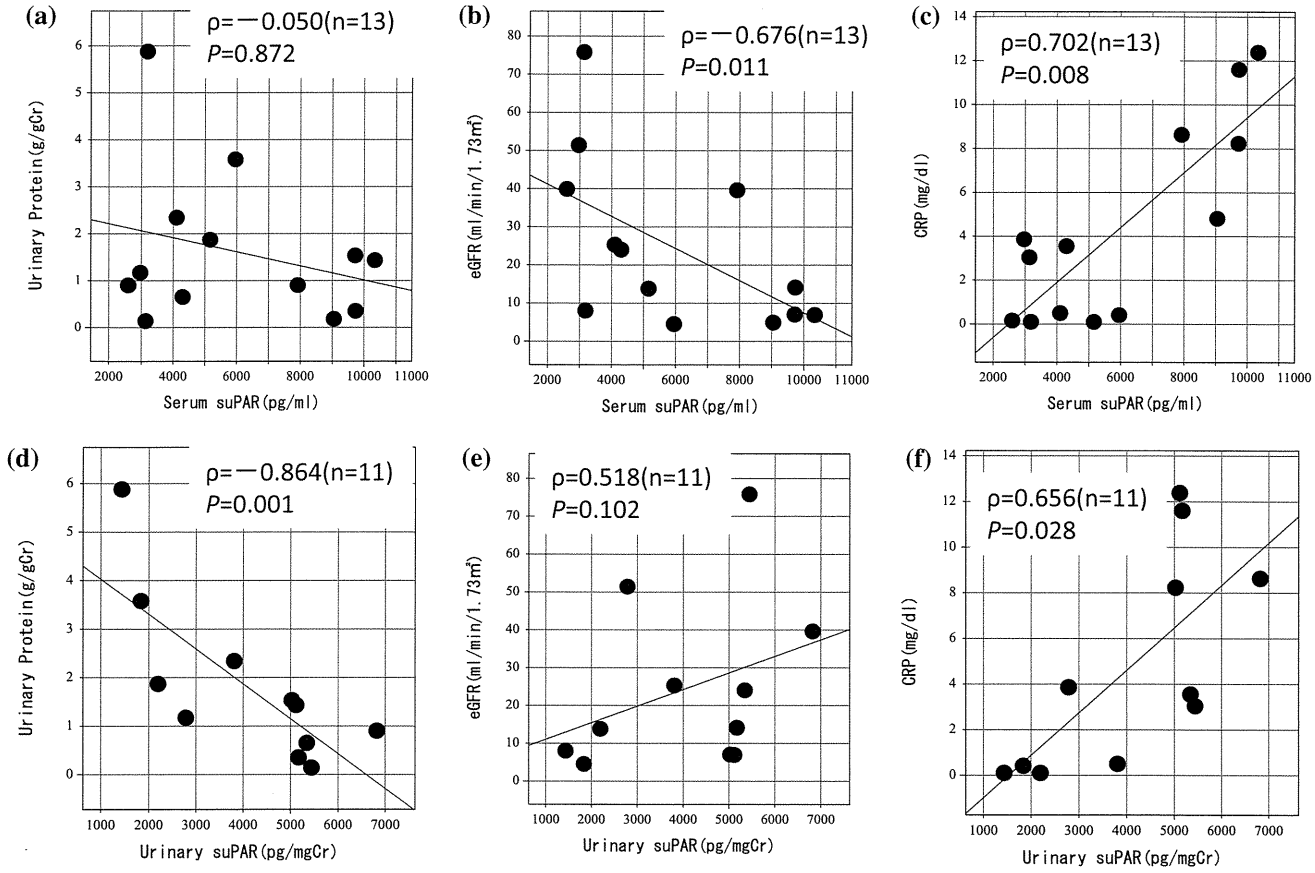

Fig. 3 The correlations between serum or urinary suPAR levels before therapy and the clinical parameters in ANCA-GN. **a** Serum suPAR and urinary protein, **b** serum suPAR and eGFR, **c** serum suPAR and CRP, **d** urinary suPAR and urinary protein, **e** urinary suPAR and eGFR, **f** urinary suPAR and CRP

within 2 months after therapy by using UP at 2 months after therapy (2M UP), s-suPAR at 2 months after therapy (2Ms-suPAR) or changes in u-suPAR during 2 months after therapy (Δ2M u-suPAR) (Table 2).

The most useful parameter for differentiating FSGS from MCNS

Comparisons of the areas under the curves of receiver operating characteristic analyses (AUC-ROCs) of clinical parameters in differentiating FSGS from MCNS are shown in Table 2. To differentiate FSGS from MCNS, Δ2M s-suPAR was found to be the most useful predictor, whereas UP was not (The cut-off values shown in Table 2, and ROC curves of suPAR and UP in supplement figures 3, 4.)

Clinical severity of and crescentic formation in ANCA-GN were associated with s-suPAR

In the ANCA-GN group, a significant positive correlation was noted between s-suPAR before immunosuppressive therapy and clinical severity ($n = 13$, $\rho = 0.651$, $p = 0.016$) or the percentage of crescentic formation ($n = 10$, $\rho = 0.770$, $p = 0.009$), whereas no correlation was noted between pretreatment u-suPAR and clinical severity ($n = 12$, $\rho = 0.300$, $p = 0.344$) or the percent of crescentic formation ($n = 9$, $\rho = 0.150$, $p = 0.700$).

Discussion

This single-center retrospective cohort study clarified the clinical significance of s- and u-suPAR in Japanese untreated primary NS and ANCA-GN patients. This study assessed u-suPAR in Japanese adult patients with NS for the first time. Pretreatment s-suPAR was significantly higher in the primary NS and ANCA-GN groups, whereas no significant differences were noted among the disease types of primary NS, suggesting that FSGS cannot be differentiated from other disease types of primary NS based on pretreatment s-suPAR. S-suPAR was previously shown to be significantly higher in primary FSGS only [1, 4], whereas, similar to our results, no significant differences were noted between FSGS and other types of

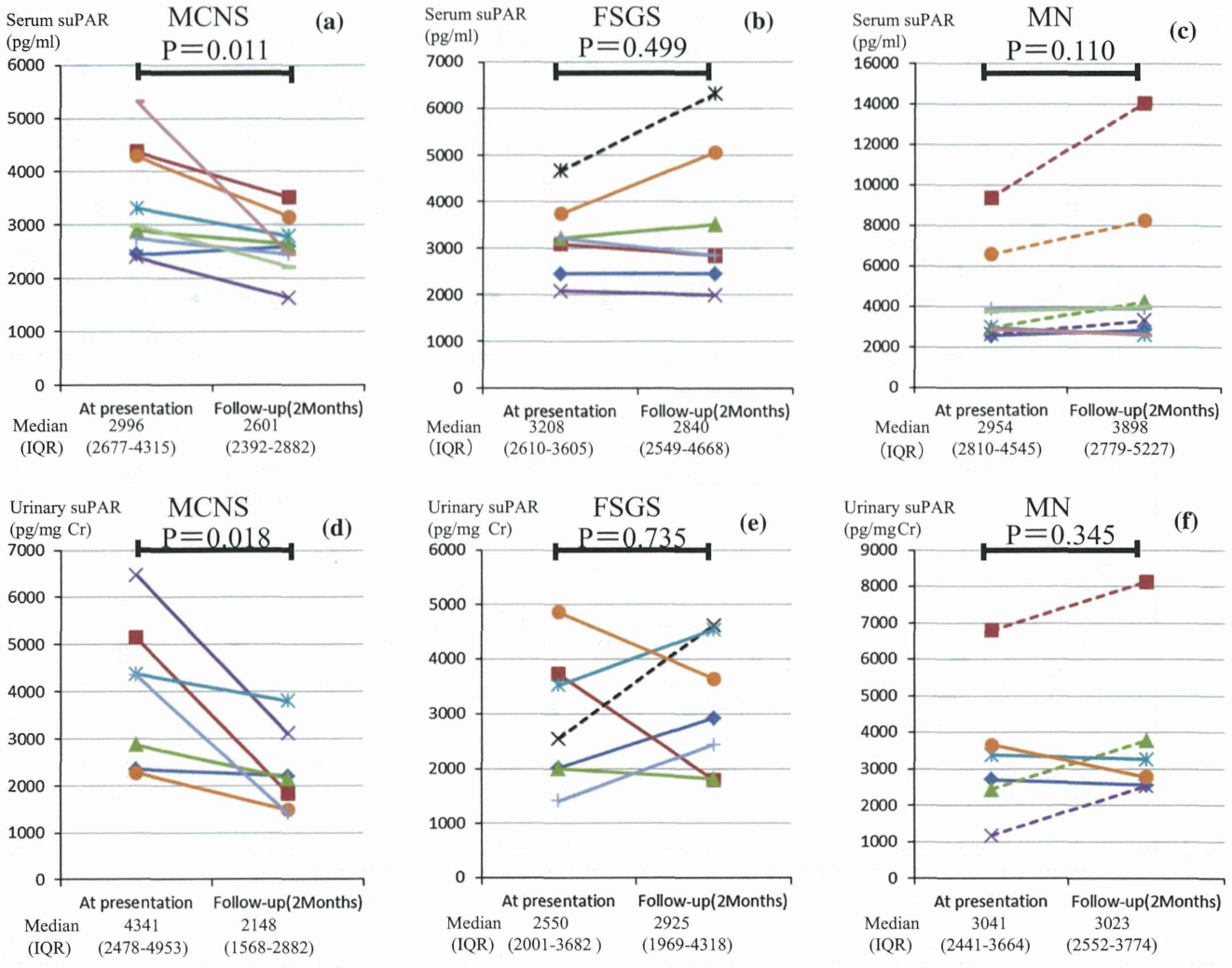

Fig. 4 The changes of serum and urinary suPAR levels after therapy among the disease types of primary NS *Solid lines* are non-refractory cases. *Dotted lines* are the refractory cases. In patients with MCNS (**a**) (**d**), serum and urinary suPAR levels significantly decreased. In patients with FSGS (**b**) (**e**) and MN (**c**) (**f**), serum and urinary suPAR levels did not change

primary NS by other studies [6–8, 12, 13]. The influence of immunosuppressive therapy on s-suPAR was considered to be the cause of this inconsistency [3, 4, 14, 15]. However, as shown in our results, comparisons of pretreatment s-suPAR, which may be specific to each disease, revealed no significant differences among the diseases.

Since the molecular weight of the main fragment of suPAR is 22 kDa and it passes through the glomerular filtration barrier, s-suPAR may be influenced by GFR or increase due to nonspecific inflammation [16–18]. No significant differences were noted in eGFR or CRP before therapy among the disease types of primary NS, suggesting that these did not influence the comparison of pretreatment s-suPAR in this study. On the other hand, pretreatment eGFR was lower and pretreatment CRP was higher in the ANCA-GN group than in the primary NS group, suggesting that renal dysfunction and inflammation led to s-suPAR being higher in the ANCA-GN group than in the primary NS group. We then investigated the relationships between pretreatment s-suPAR and clinical parameters. A strong inverse correlation was noted between s-suPAR and eGFR in FSGS, as previously reported [3, 4]. Furthermore, an inverse correlation with eGFR was also found in all primary NS and MN, which was consistent with recent findings [6–8]. In contrast, no correlation with any of the clinical parameters was noted in MCNS. These findings may suggest that s-suPAR is associated with renal dysfunction in primary NS, particularly in FSGS and MN patients with a poor renal prognosis.

On the other hand, s-suPAR was positively correlated with CRP and inversely correlated with eGFR in the ANCA-GN group. CRP and eGFR were identified as