Table 3 Clinical predictors for a 50 % increase in serum creatinine from the baseline level in the Cox–hazard model

Predictors	Univariate model		Multivariate model[a]	
	HR (95 % CI)	p value	HR (95 % CI)	p value
At 1 year				
Category of proteinuria[b]				
Disappeared[c]	0.07 (0.01–0.33)	0.001[#]	0.06 (0.01–0.57)	0.014[#]
Mild[c]	0.10 (0.12–0.80)	0.030[#]	0.02 (0.00–0.29)	0.003[#]
Moderate[c]	0.55 (0.16–1.98)	>0.2	0.24 (0.04–1.25)	0.089
U-RBC <5/hpf[d]	2.59 (0.71–9.42)	0.148	–	–
Clinical remission[d]	0.35 (0.08–1.57)	0.170	–	–
At baseline				
Age (years)	1.04 (0.99–1.08)	0.092	1.00 (0.94–1.06)	>0.2
Female[d]	1.06 (0.36–3.16)	>0.2	–	–
Current smoking[d]	3.96 (1.33–11.8)	0.013[#]	1.27 (0.28–5.58)	>0.2
BP ≥130/80 mmHg[d]	1.31 (0.36–4.79)	>0.2	–	–
UPE (g/day)	2.09 (1.43–3.07)	<0.001[#]	–[e]	–[e]
U-RBC ≥30/hpf[d]	0.22 (0.06–0.79)	0.021[#]	0.34 (0.06–1.99)	>0.2
eGFR <60 ml/min/1.73 m^2 [d]	11.5 (2.55–52.3)	0.002[#]	24.3 (2.72–217)	0.004[#]
Concurrent treatment				
Tonsillectomy[d]	0.37 (0.11–1.21)	0.099	1.23 (0.27–5.55)	>0.2
RAAS inhibitors[d]	2.06 (0.67–6.29)	>0.2	–	–

HR hazard ratio, *CI* confidence interval, *UPE* urinary protein excretion, *U-RBC* urinary sediments of red blood cells, *NE* not enrolled in the multivariate model, *eGFR* estimated glomerular filtration rate, *RAAS* renin–angiotensin–aldosterone system

[a] If the p value of the variable was <0.1 in the univariate model, the predictor was selected for the multivariate model

[b] The category is shown in Table 2

[c] Reference = *Severe* category

[d] Yes versus no

[e] As it was related to category of UPE at 1 year (see Table 2), it was not enrolled in the multivariate model

[#] $p < 0.05$

Table 4 Pathological predictors and UPE <0.4 g/day at 1 year for a 50 % increase in the serum creatinine level from baseline in the Cox model

Predictors	Univariate model		Multivariate model A		Multivariate model B	
	HR (95 % CI)	p value	HR (95 % CI)	p value	HR (95 % CI)	p value
Oxford classification						
M1 versus M0	0.93 (0.24–3.61)	>0.2	–	–	–	–
E1 versus E0	0.23 (0.06–0.89)	0.033[#]	0.44 (0.10–1.91)	>0.2	–	–
S1 versus S0	2.03 (0.26–16.0)	>0.2	–	–	–	–
T1 versus T0	6.97 (1.66–29.2)	0.008[#]	4.35 (1.02–18.5)	0.047[#]	–	–
T2 versus T0	12.8 (2.12–77.1)	0.005[#]	19.1 (2.55–144)	0.004[#]	–	–
Ext, present versus absent	0.44 (0.09–2.06)	>0.2	–	–	–	–
HG						
HG1 versus HG3 + 4	0.00 (0.00–100<)	>0.2	–	–	0.00 (0.00–100<)	>0.2
HG2 versus HG3 + 4	0.24 (0.06–0.92)	0.038[#]	–	–	0.36 (0.08–1.51)	0.161
UPE at 1 year <0.4 g/day[a]	0.10 (0.03–0.36)	<0.001[#]	0.08 (0.01–0.45)	0.004[#]	0.06 (0.01–0.29)	0.001[#]

HR hazard ratio, *CI* confidence interval, *M* mesangial hypercellularity, *E* endocapillary hypercellularity, *S* segmental sclerosis, *T* tubulointerstitial atrophy/fibrosis, *Ext* extracapillary lesion, *HG* histological grade, *UPE* urinary protein excretion volume

[#] $p < 0.05$

[a] Yes versus no

steroid therapy: five bacterial infections (tonsillitis, pharyngitis) and two viral infections (influenza). Two females became pregnant during the follow-up and maintained a stable renal function.

Discussion

The goal of this study was to identify the level of proteinuria after steroid therapy associated with a favorable renal outcome in IgAN patients. Previous studies by Reich et al. [4], Hwang et al. [5], or Le et al. [6] have demonstrated that the average level of proteinuria during the whole period of follow-up (A-P) was significantly associated with the renal outcome, providing a targeted proteinuria during long-term follow-up. In contrast, we identified a therapeutic indicator of a favorable renal outcome as an early response to the steroid therapy, which might be more practical than A-P, whereas it was not analyzed in the previous studies. We adopted 1 year as the time to assess the attenuated proteinuria, since another Cox model in our cohort revealed that the values for proteinuria at 1 year were significantly associated with the outcome, whereas those at baseline or 6 months were not (data not shown).

In this study, the spline model revealed that the threshold UPE predicting the outcome was approximately 0.4 g/day. In addition, a multivariate Cox model including the categorized UPE at 1 year revealed that not only the *Disappeared* category but also the *Mild* category were significantly associated with favorable renal survival relative to the *Severe* category. Therefore, attenuated proteinuria <0.4 g/day at 1 year after treatment can lead to a favorable outcome, as well as the disappearance of proteinuria. The predictive power of UPE <0.4 g/day at 1 year for renal survival was confirmed even after adjusting for pathological predictors determined by the multivariate model (Table 4).

Concerning the impact of clinical remission at an early phase on the renal outcome, Tatematsu et al. [20] showed that clinical remission within 2 years after 6 months of steroid therapy was associated with limiting the eGFR decline. In contrast, clinical remission at 1 year was not significantly associated with the endpoint in our univariate Cox model (Table 3). Although the reasons for the discrepancy between the two studies are unknown, there might be several factors responsible. For example, the timing for assessment of clinical remission was different: during the first 2 years in Tatematsu's study and at 1 year after the intervention in our study. Furthermore, the fact that the incidence of the endpoint in our patients achieving clinical remission at 1 year after the therapy was not significantly different from that in those without clinical

remission (4.1 vs. 12.0 %, respectively, $p > 0.2$) may have affected the results shown in Table 3.

Our retrospective study has several limitations. First, we did not include control patients who were followed by supportive therapy alone. Second, the study population and statistical power were small, and the observation period was relatively short to evaluate the outcome in IgAN, leading to the small number of outcomes. Since a limited number of outcomes would generally restrict the number of explanatory variables in multivariate models, we additionally tested the Cox–hazard model for the outcome with two explanatory variables: UPE at 1 year <0.4 g/day and propensity score. The propensity model for UPE at 1 year <0.4 g/day was constructed with the baseline characteristics or pathological parameters. After adjusting the propensity score, we also found the predictive power of UPE at 1 year <0.4 g/day for the outcome (data not shown), suggesting the consistency of the significance of UPE at 1 year <0.4 g/day. Nevertheless, the value of UPE at 1 year <0.4 g/day as a favorable predictor should be ascertained in other studies with longer observation periods and a larger number of outcomes. Third, the role of recurrent proteinuria after 1 year on the progression of IgAN should be examined, since clinical remission was not associated with the endpoint in this study.

In conclusion, the achievement of proteinuria <0.4 g/day at 1 year after 6 months of steroid therapy is an optimal goal for achieving a subsequent favorable renal survival, independent of the baseline renal function or renal pathological changes. Further investigations of the impact of recurrence during follow-up on the endpoint are now in progress.

Conflict of interest   None.

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Clinical findings on ANCA-associated renal vasculitis from the Japan RPGN registry obtained via a questionnaire survey

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Abstract Renal involvement with significant organ damage is common in anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV). As a result, it is independently referred to ANCA-associated renal vasculitis. Clinically, ANCA-associated renal vasculitis is characterized by rapidly progressive glomerulonephritis. Pathologically, it is defined by pauci-immune type necrotizing and crescentic glomerulonephritis. According to previous reports from all over the world, the etiology, prevalence, and prognosis of RPGN including ANCA-associated renal vasculitis varies among races and periods. To elucidate the clinical characteristics of Japanese RPGN patients, a registry derived from a questionnaire survey was

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established in 1999 and maintained until 2006. As a result, 1,772 cases were collected, analyzed, and reported previously. In this mini-review, we outline the characteristic clinical findings of Japanese patients (Asian) with ANCA-associated renal vasculitis, based on the registry data.

Keywords ANCA-associated renal vasculitis · RPGN · Japan · Registry · Questionnaire survey

Clinical findings of ANCA-associated renal vasculitis and RPGN in Japan

The frequency of renal involvement and rapidly progressive glomerulonephritis (RPGN) in Japanese patients with anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) is still unclear. A Japan RPGN registry derived from a questionnaire survey was established in

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Table 1 Number of patients with RPGN. This table was prepared with partial modification [1]

Diagnosis	Classification	Total RPGN cases	
		n	%
Primary			
Crescentic GN	Anti-GBM antibody-associated crescentic GN	81	4.6
	Immune-complex-associated crescentic GN	35	2.0
	Renal-limited vasculitis	745	42.0
	Overlapped crescentic GN	31	1.7
	Undifferentiated primary crescentic GN	28	1.6
Primary GN with crescents	Mesangioproliferative glomerulonephritis	15	0.8
	Membranous nephropathy	5	0.3
	IgA nephropathy	43	2.4
	Non-IgA mesangial proliferative GN	8	0.5
	Other primary GN	3	0.2
Systemic disease-associated			
	Goodpasture's syndrome	27	1.5
	Systemic lupus erythematosus	66	3.7
	Granulomatosis with polyangiitis (Wegener's)	46	2.6
	Microscopic polyangiitis	344	19.4
	Other necrotizing vasculitis	15	0.8
	Purpura nephritis	36	2.0
	Cryoglobulinemia	12	0.7
	Rheumatoid arthritis	24	1.4
	Malignant neoplasm	3	0.2
	Other systemic diseases	40	2.3
Infection-associated			
	Poststreptococcal acute glomerulonephritis	10	0.6
	Abscess	6	0.3
	Hepatitis C virus	2	0.1
	Other infectious diseases	20	1.1
Drug-associated		10	0.6
Others		17	1.0
Unknown		100	5.6
Total		1772	100.0

1999 and maintained until 2006. As a result, 1772 cases were collected, analyzed and reported [1, 2]. The clinical entity of RPGN is shown in Table 1 [1, 2]. Pauci-immune-type renal-limited vasculitis was the most frequently observed clinical entity of RPGN (42.0 %). Among patients with renal-limited vasculitis (RLV), myeloperoxidase (MPO)-ANCA-associated cases made up 88.1 % and proteinase 3 (PR3)-ANCA-associated cases made up 7.4 %. Among cases of microscopic polyangiitis (MPA), which was the second most common clinical entity of RPGN (19.4 %), MPO-ANCA-associated cases made up 91.8 % and PR3-ANCA-associated cases made up 6.1 %. By contrast, in cases of granulomatosis with polyangiitis (Wegener's) occurring among Japanese individuals with RPGN (2.6 %), MPO-ANCA-associated cases made up 22.7 % and PR3-ANCA-associated cases made up 71.1 %. That is, most Japanese patients with AAV and RPGN were estimated to be positive for MPO-ANCA. Additionally, the age distribution of Japanese RPGN was a characteristic finding [1]. Among all RPGN subjects, the mean age at presentation significantly increased during the observation period. The main reason for this secular change was a significant increase in the mean age of subjects with RLV (61.85–67.28 years), MPA (64.60–68.77 years), and anti-GBM antibody-mediated RPGN (52.05–61.59 years) in recent years. This increase in the age of the onset of RPGN seems to reflect the longevity of the Japanese population and the aging of Japanese society.

Next, the speed of renal deterioration in this RPGN survey was also examined. Because RPGN is a loosely-defined term

Table 2 The clinical grading system for predicting RPGN patient prognosis [1]

Clinical score	Serum creatinine (mg/dl)	Age (years)	Lung involvement	Serum CRP (mg/dl)
0	<3	≤59	Negative	<2.6
1	3–6	60–69		2.6–10.0
2	≥6	≥70	Positive	>10.0
3	Dialysis			
Clinical grade				
I				0–2
II				3–5
III				6–7
IV				8–9

Fig. 1 Clinical grading system for predicting patient prognosis. A clinical grading system was applied to all RPGN patients. This figure was prepared with partial modification [1]

and is challenging to define, we need to provide specific data for an established definition. Seventy-eight cases in which diffuse crescentic glomerulonephritis was confirmed on renal biopsy were selected and analyzed. In cases with RPGN, the average speed of the increase in the serum creatinine level was 1.03 mg/dl per week and the decrease in the estimated glomerular filtration rate (GFR) was 4.6 ml/min/1.73 m^2 (18.5 %) per week. Moreover, in 52 cases with MPO-ANCA-associated RPGN, the average speed of the increase in the serum creatinine level was 0.80 mg/dl per week, and the decrease in the estimated GFR was 3.6 ml/min/1.73 m^2 (16.6 %) per week. The Birmingham Vasculitis Activity Score (BVAS), a popular vasculitis activity score, has adopted the following assessment criteria of renal impairment, as specified by professional opinion: an increase in serum creatinine of more than 30 % or a decrease in creatinine clearance of more than 25 % within 4 weeks (personal communication with Professor RA Luqmani) [3]. The definition of RPGN varies among different countries of the world, and a universal standard definition of RPGN should be established in the future.
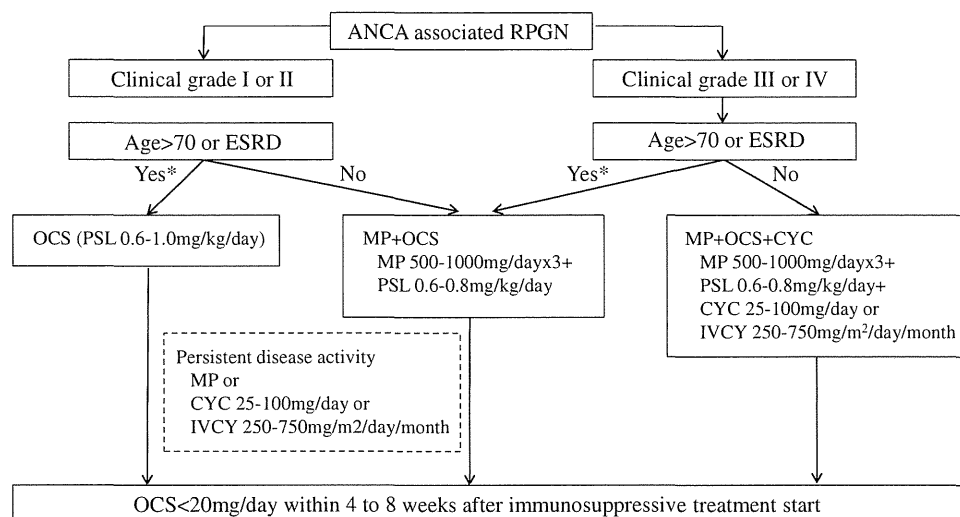
The first version of the clinical guidelines for Japanese RPGN was published in 2002, and the second version was published in 2011; these were based on the Japan RPGN registry established using a questionnaire survey (articles in Japanese). A clinical degree of severity that was calculated using four items, namely serum creatinine level, age, lung involvement, and serum C-reactive protein level, was defined in these clinical guidelines (Table 2). This was well correlated with the life prognosis of all patients with RPGN and MPO-ANCA-associated RPGN (Fig. 1). Moreover, a therapeutic algorithm for ANCA-associated RPGN based on the clinical degree of severity was suggested (Fig. 2) [2]. This clinical grading system was able to estimate the prognosis in cases of ANCA-associated RPGN and provided an approach to the classification of the treatment choices. In a recent report, the authors named this
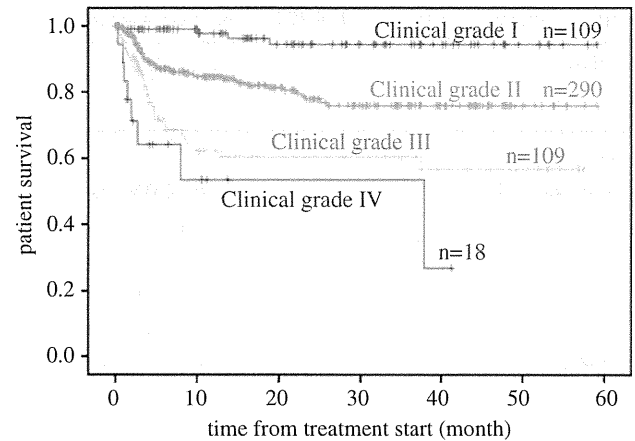
algorithm the Japanese Vasculitis Activity Score (JVAS), and it was found to be useful as a method of grading activity in cases of AAV in comparison with BVAS [4]. It was positively correlated with BVAS. However, at this time, the clinical degree of severity is consistently used as an index of life prognosis in ANCA-associated RPGN. If this clinical grading system is to be applied to the vasculitis activity score, additional investigations are needed.

Because of the publication of the clinical guidelines for Japanese RPGN in 2002, the prognosis for Japanese RPGN including AAV was markedly improved recently [1]. Standard induction therapy consisted of both corticosteroids and cyclophosphamide in Europe, but the clinical guidelines for Japanese RPGN adopted an independent therapeutic algorithm for ANCA-associated RPGN because of the high prevalence of elderly patients as mentioned above. According to the analysis of the Japan RPGN registry, infection was a major cause of death [1]. During the observation period, 31.8 % of patients died within 0–98.8 months. In recent years, the mortality rate decreased from 38.7 % (between 1989 and 1998) to 18.0 % (between 2002 and 2007). By contrast, the rate of infection as a cause of death was not decreased, from 48.1 % (between 1989 and 1998) to 55.9 % (between 2002 and 2007). Infection as the cause of death was frequent in the early phase of treatment. Therefore, the avoidance of severe adverse effects including infections became a priority in Japan, and milder treatment was chosen in the therapeutic regimen. As a result of this change, the life prognosis and renal survival of all RPGN patients were undoubtedly improved [2]. Additionally, the life prognosis and renal survival of patients with MPO-ANCA-associated RPGN were also improved. In contrast, the reduction in the use of immunosuppressant reagents increased the rate of relapse in patients with MPO-ANCA-associated RPGN.

Fig. 2 Treatment algorithm for ANCA-associated RPGN in Japan [2]. *ESRD* end-stage renal disease, *OCS* oral corticosteroid, *MP* methylprednisolone, *PSL* prednisolone, *CYC* cyclophosphamide, *IVCYC* intravenous cyclophosphamide

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                          ┌─────────────────────────┐
                          │   ANCA associated RPGN  │
                          └─────────────────────────┘
             ┌──────────────────────┐        ┌──────────────────────┐
             │  Clinical grade I or II │      │ Clinical grade III or IV │
             └──────────────────────┘        └──────────────────────┘
             ┌──────────────────────┐        ┌──────────────────────┐
             │    Age>70 or ESRD     │        │    Age>70 or ESRD     │
             └──────────────────────┘        └──────────────────────┘
              Yes*            No                Yes*            No
```

| OCS (PSL 0.6-1.0mg/kg/day) | MP+OCS
MP 500-1000mg/dayx3+
PSL 0.6-0.8mg/kg/day | MP+OCS+CYC
MP 500-1000mg/dayx3+
PSL 0.6-0.8mg/kg/day+
CYC 25-100mg/day or
IVCY 250-750mg/m²/day/month |

Persistent disease activity
MP or
CYC 25-100mg/day or
IVCY 250-750mg/m2/day/month

OCS<20mg/day within 4 to 8 weeks after immunosuppressive treatment start

*Older patients often suffered from opportunistic infection. Milder treatment (less dose of PSL, without MP or CYC were recommended.

Therefore, it became important to establish maintenance therapy for MPO-ANCA-associated RPGN as quickly as possible. In Japan, a randomized controlled trial of a maintenance therapy using a milder immunosuppressant drug, mizoribine, for MPO-ANCA-associated RPGN is currently underway [Mizoribine for ANCA RPGN Relapse-Prevention Study (MARPGN study)]. A total of 44 cases had been enrolled as of December, 2011, at which point the entry of new patients into the study was ended. The rate of relapse and the effectiveness of mizoribine for maintenance therapy are expected to be determined in this study.

In the present article, we reviewed the clinical findings of ANCA-associated renal vasculitis in Japan. The Japan RPGN registry, based on a questionnaire survey, and the establishment of independent clinical guidelines have definitely improved the medical practice involved in the treatment of Japanese patients with AAV. A comparative discussion regarding the Japanese clinical guidelines and global guidelines is now needed.

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Evaluation of the newly proposed simplified histological classification in Japanese cohorts of myeloperoxidase-anti-neutrophil cytoplasmic antibody-associated glomerulonephritis in comparison with other Asian and European cohorts

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Abstract The prognostic value of renal biopsy in anti-neutrophil cytoplasmic antibody (ANCA)-associated glomerulonephritis is widely recognized; however, there is no consensus regarding its pathological classification. Berden et al. proposed a new classification of glomerulonephritis in ANCA-associated vasculitis (AAV) categorized into focal, crescentic, mixed, and sclerotic classes and showed its prognostic value in 100 international multicenter cohorts for 1- and 5-year renal outcomes. In order to evaluate whether this new classification has predictive value and reproducibility in Japanese AAV cases, 87 cohorts with only microscopic polyangiitis in 3 limited centers in Japan were analyzed. In addition, those from Japan, Europe (Berden's cohorts) and China were compared in a recent report.

Introduction

We recently proposed pathological parameters of renal lesions observed in anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) patients [1]. The purpose of this proposal was (1) standardization of pathological findings in AAV should be authorized in Japan; (2) comparison with the European Vasculitis Study Group (EUVAS) standardization should be available; and (3) pathological parameters correlated with specific clinical findings should be evaluated (Table 1). As a result, the pathological parameters selected were almost compatible with those selected by EUVAS except for the collapse of glomeruli as the chronicity parameter; however, further evaluation using these parameters to investigate potential markers for the probability of end-stage renal disease (ESRD) is needed.

Among the parameters listed above, the number of normal or sclerotic glomeruli was proved substantially to be a prognostic indicator of renal outcome in accordance with basal renal function [2–4]; however, no sufficient consensus exists regarding the pathological classification. Recently, using some of the glomerular parameters, an international working group of renal pathologists proposed a new histopathological classification of glomerulonephritis (GN) in

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Table 1 Pathological parameters nominated for evaluation of active and chronic lesion in ANCA-related vasculitis in Japan (comparable with EUVAS)

Glomerular lesion	
No. of normal glomeruli	
Active lesion	Chronicity lesion
Mesangial proliferation	Sclerotic lesion
Endocapillary hypercellularity	Global sclerosis
Tuft necrosis	Segmental sclerosis
Cellular, fibrocellular crescent formation	Fibrous crescent
<50 %	<50 %
>50 %	>50 %
Rupture of Bowman's capsule	Adhesion
	Collapse[a]
Tubulointerstitial lesion	
Active lesion	Chronicity lesion
Tubulitis	Atrophic tubule
Disruption of tubular basement membrane	Interstitial fibrosis
Interstitial cell infiltration	
Granulomatous lesion	
Peritubular capillaritis[a]	
Vascular lesions	
Active lesion	Chronicity lesion
Necrotizing	Arteriosclerosis
Endoarteritis	
Cell infiltration	
Thromboembolism	
Granulomatous lesion	

[a] Parameter not nominated in EUVAS

AAV with four categories (focal, crescentic, mixed and sclerotic), corresponding to the severity of renal function loss in this order during a 5-year follow-up [5]. As the evaluation was performed in 100 cases, consisting of 39 cases of granulomatosis with polyangiitis (GPA) and 61 cases of microscopic polyangiitis (MPA) in 32 centers in 9 European counties, the influence of the relatively mixed races and disease types could not be excluded. In Japan, >90 % of ANCA-positive GN is diagnosed as MPA, in which renal involvement is more frequent than in GPA, as previously reported [6]. In this study, we evaluated the predictive potential of this newly proposed categorization in myeloperoxidase (MPO)-ANCA-dominant MPA patients in Japan.

Patients and methods

Eighty-seven patients with primary systemic vasculitis, in accordance with the Chapel Hill consensus criteria [7], diagnosed and treated from 2001 to 2010 in three centers (Kitano Hospital in Osaka, Tokyo Women Medical College

Table 2 Comparison among evaluations of GN histological categories with clinical background in Europe, China and Japan

	European [5]	Japan	China [8]
Patients (number)	100	87	121
Centers (number)	32	3	1
Median age (range)	62.6 (20–80)	63.0 (17–85)	57.2 (15–81)
Male to female (number)	54:46	37:50	64:57
Clinical diagnosis (%)			
GPA	39 (39)	0	49 (40.5)
MPA	61 (61)	87 (100)	68 (56.2)
Renal-limited vasculitis	0	0	4 (3.3)
ANCA test (indirect immunofluorescence or ELISA)			
PR3-ANCA	45	0	13
MPO-ANCA	47	76	108
ANCA(−)	2	0	0
Missing	3	11	0
Median number of glomeruli per biopsy (range)	14.8 (10–49)	26.5 (10–98)	25.7 (NS)
Pathological classification number (%)			
Focal	16 (16)	40 (46.0)	33 (27.3)
Crescentic	55 (55)	7 (8.0)	53 (43.8)
Mixed	16 (16)	26 (29.9)	24 (19.8)
Sclerotic	13 (13)	14 (16.1)	11 (9.1)
Serum creatinine (mg/dl)			
Focal	NS	1.51 ± 1.49	2.22 ± 1.90
Crescentic		2.42 ± 1.67	5.01 ± 2.73
Mixed		3.37 ± 3.17	3.86 ± 2.69
Sclerotic		7.52 ± 4.92	8.51 ± 3.42
Death at 1-year follow-up	25/100	11/84	NS
Renal survival at 1-year follow-up			
Focal, crescentic, mixed, sclerotic (%)	93, 84, 69, 50	100, 86, 96, 35	100, 73, 83, 29
Renal survival at 5-year follow-up			
Focal, crescentic, mixed, sclerotic (%)	93, 76, 61, 50	100, 86, 96, 29	NS

Data of three patients were lost due to transfer to different hospitals before 1-year follow-up

NS not shown in the report

in Tokyo and Shimoshizu National Hospital in Chiba) were analyzed. In all cases, renal biopsy was performed before treatment. Specimens including a minimum of 10 whole glomeruli were enrolled. Hematoxylin and eosin, methenamine silver, periodic acid-Schiff, and Masson trichrome staining were used for evaluation. The histological categorization based on glomerular lesion was performed following Berden's group [5]—focal ≥50 % normal glomeruli, crescent ≥50 % of glomeruli with cellular crescents, sclerotic ≥50 % of glomeruli with global sclerosis, and mixed <50 % normal, <50 % crescentic, <50 %

185

globally sclerotic glomeruli. A minimum of 6 months prognosis was observed for all patients. Renal and life survivals were analyzed at onset, 6 months, 1 year and 5 years after renal biopsy in available patients (87 at onset and 6 months, 84 at 1 year, 78 at 5 years).

Results

Patient profile and outcome in Japanese cohort

Median age was almost identical to the European study; however, males were dominant in Japan in contrast to a slight female dominance in Europe (Table 2).

All cases in Japan had MPA; MPO-ANCA was positive in 76/87 (87.3 %). The median glomerular number was 26.5 in Japanese samples. At 6 months follow-up, 11 patients reached ESRD and a further 8 patients had died. At 1-year follow-up, no more patients had reached ESRD and a total of 11 patients had died. At 5-year follow-up, 18 patients had died and another 12 patients had reached ESRD.

Classification of the renal biopsy in Japanese cohorts

In Japanese patients, almost half of the cases were categorized as focal (40/87; 46.0 %) with 14/87 (16.1 %) as sclerotic. Of the other 32 cases, only 7 (8.0 %) were categorized as crescentic, with the remaining 26 cases (29.9 %) being classed as mixed. As shown in Fig. 1, the Kaplan–Meier curve at the 5-year follow-up showed no increase of probability to ESRD in focal cases and a low increase in mixed cases; however, this increased with the ascending categories of crescentic and sclerotic GN.

Comparison among evaluations of GN histological categories in Europe, China and Japan

The predictive value and reproducibility of this new classification from Japan, Europe and China were compared in a recent report [8]. As shown in Table 2, among the 100 respective patients (32 centers; Europe), 121 (1; China) and 87 (3; Japan), the GPA:MPA ratio was similar between Europe and China (39:61 and 49:64) in contrast to all MPA (0:87) in Japan. On the other hand, for serum ANCA positivity, MPO-ANCA positivity was dominant in China (89.1 %) and Japan (87.4 %) compared to Europe (45 %), where there was relatively high PR3-ANCA positivity (47 %) compared with China and Japan (10.7 and 0 %, respectively). The average numbers of glomeruli per case were significantly higher both in Japan (26.5) and China (25.7) than in Europe (14.8). The distribution of the four histological categories of GN were similar in Europe and
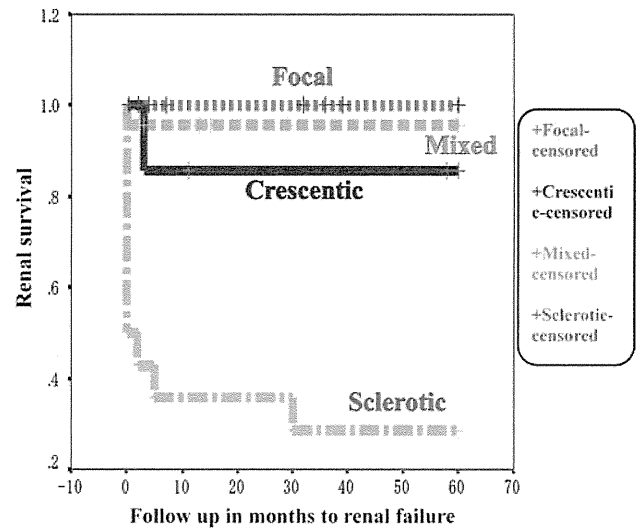

Fig. 1 Renal survival (no development of end-stage renal failure) according to the four histologic categories in Japanese cohorts

China with crescentic cases being dominant (55 and 47 %, respectively), whereas in Japan, the number in this category was significantly lower (8.0 %). The probability of developing ESRD increased with the ascending categories of focal, crescentic, mixed, and sclerotic in Europe, and focal, mixed, crescentic and sclerotic in China. In Japan, as mentioned above, there was no increase of probability to ESRD in focal and mixed, but there was a high increased in sclerotic, as in Europe and China.

Discussion

The histopathological findings of AAV in the kidney are considered to show a variety of lesions, of which crescentic and/or focal necrotizing GN as well as small-vessel arteritis are the most prominent [7]. In addition to the baseline laboratory data concerning renal lesions such as hematuria, proteinuria and decreased estimated glomerular filtration rate with systemic inflammatory signs such as C-reactive protein and organ involvement symptoms such as hemoptysis, renal histological findings have been expected to give highly reliable information not only to select the treatment protocol but to predict the outcome at baseline. Trials for the global standardization of active and chronic pathological parameters specifically in AAV have been performed not only in EUVAS but also in Japan, where a higher prevalence of MPA than EUVAS has been recognized, although the AAV prevalence itself is almost the same [9]. As shown in Table 1, these parameters are common findings in AAV. Almost all parameters are common in EUVAS selection, so our Japanese standardization of clinicopathologically critical parameters in AAV seems to be globally fulfilled.

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The new classification of GN into four categories (focal, crescentic, mixed, sclerotic) by selecting some of the parameters of Berden et al. [5] was highly predictive in AAV patients from multicenters in Europe. In Japan, the significantly lower frequency of crescentic and relatively higher frequency of focal cases were noted; this might be partly attributed to the earlier intervention of renal biopsy after discovering a urinary or renal function abnormality in Japan. The relatively low creatinine level of the focal group in Japan compared with that of the same group in China might support this tendency. As the progression of renal injury tends to be different between MPA and GPA, comparisons should be performed only between MPA in Europe and in Japan. This was not possible in this classification study because there were no data on the ratio of MPA in the crescentic group in Europe. In this study, the Kaplan–Meier curve revealed the highly favorable prognosis of the mixed group. This indicates that the prognosis of this group is attributed to additional pathological parameter such as tubulointerstitial or vascular lesions nominated previously in Europe and Japan. At present, at least for MPA-oriented cohorts in Japan, this classification only by glomerular parameters might be insufficient to predict the probability of progressing to ESRD.

The comparison of European, Japanese and Chinese cohorts would be highly informative. The similarity of the GPA/MPA ratio between Europe and China in contrast to that of MPO-ANCA dominancy between Japan and China indicates that many GPA are MPO-ANCA-positive in China, as Chinese authors have stated. The GPA dominancy might be attributed partly to the localization of the center at a high latitude, which has been reported to be related to the high prevalence of GPA [10]. Although the numbers in the four categories were similar between Europe and China, there was a difference in the order of the increase of probability of progressing to ESRD between mixed and crescentic. The significantly more favorable prognosis of mixed than crescentic in China is similar to Japan, where both focal and mixed rarely showed progress to ESRD.

In conclusion, the mixed group in the new classification has high heterogenicity of histological activity and chronicity, which shows the insufficiency of this classification for prediction of the probability of progressing to ESRD. Re-evaluation of the predictive value by adding other parameters such as interstitial or vascular lesions for MPA-oriented cohorts is expected.

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clinical investigation

A multicenter cross-sectional study of circulating soluble urokinase receptor in Japanese patients with glomerular disease

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Elevated serum-soluble urokinase receptor (suPAR) levels have been described in patients with focal segmental glomerulosclerosis (FSGS) in several different cohorts. However, it remains unclear whether this is the case for Japanese patients and whether circulating suPAR can be clinically useful as a diagnostic marker. To determine this, we measured serum suPAR levels in 69 Japanese patients with biopsy-proven glomerular diseases in a cross-sectional manner. The serum suPAR levels showed a significant inverse correlation with renal function by univariate (R^2 of 0.242) and multivariate ($\beta = 0.226$) analyses. Even after excluding patients with renal dysfunction, no significant difference in the suPAR levels was detected among the groups. Receiver operating characteristic analysis and measures of the diagnostic test performance showed that suPAR was not a useful parameter for differentiating FSGS from the other glomerular diseases (AUC-ROC: 0.621), although a small subgroup analysis showed that patients with FSGS, treated with steroids and/or immunosuppressants, had significantly lower suPAR levels. Patients with ANCA–associated glomerulonephritis had significantly higher levels of suPAR compared with the other disease groups, which may be owing to their lower renal function and systemic inflammation. Thus, suPAR levels are significantly affected by renal function and have little diagnostic value even in patients with normal renal function.

Focal segmental glomerulosclerosis (FSGS) is one of the leading causes of steroid-resistant nephrotic syndrome. Research on the pathogenesis of primary FSGS has been intensified because of identification of the podocyte as the major cellular target;[1] however, the disease mechanism has not been fully elucidated. One of the best-recognized notions is that some FSGS cases may be associated with a circulating factor. This concept is supported by the recurrence of FSGS soon after renal transplantation,[2,3] by the response of proteinuria to plasmapheresis[4,5] or immunoabsorption,[6,7] and by a case of nephrotic syndrome in a newborn whose mother had FSGS.[8] Recently, Gallon et al.[9] reported that reimplantation of a kidney allograft with FSGS recurrence into another patient resulted in proteinuria remission, which strongly suggested involvement of a circulating factor.

Wei et al.[10] have recently reported that soluble urokinase receptor (suPAR) was a promising candidate for circulating permeability factor by evaluating the circulating suPAR levels in sera from primary and recurrent FSGS patients. They proposed that suPAR has a causal role in the development of FSGS based on their finding that urokinase receptor (uPAR) can cause foot process effacement through the activation of β3-integrin signaling.[11] The researchers also evaluated the suPAR levels in two large cohorts of children and adults with biopsy-proven primary FSGS.[12] Although they demonstrated that the suPAR levels were significantly elevated in primary FSGS patients from both cohorts, there has been an intense

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debate over suPAR as a diagnostic marker.[13–16] Recently, Huang et al.[17] reported that the suPAR levels in primary FSGS patients have a considerable overlap with other glomerular diseases such as minimal change disease (MCD) and membranous nephropathy (MN), although these differences are significant. In a study of pediatric patients, Bock et al.[18] demonstrated that the suPAR levels in FSGS patients are even lower than those in non-glomerular kidney diseases.[18] In Japan, it is estimated that approximately 3800–4500 adults develop nephrotic syndrome annually, and primary FSGS accounts for approximately 10% of the new-onset nephrotic syndrome cases.[19] However, no data on the serum suPAR levels in Japanese FSGS patients are available yet.

In the present investigation, we performed a multicenter cross-sectional study using sera from patients with primary glomerular diseases (including FSGS) to determine whether the serum suPAR levels in Japanese patients are useful as a diagnostic marker.

RESULTS

Demographic and clinical characteristics of patients with glomerular diseases

We studied 69 serum samples from patients with biopsy-proven glomerular diseases and 17 serum samples from healthy volunteers. The subjects' demographic and clinical data are listed in Table 1. The median age of the patients was 54 years, ranging from 17 to 82 years. The patients were categorized according to the histopathological diagnoses (FSGS, MCD, IgA nephropathy (IgAN), and MN) performed by pathologists in each facility, and no overlaps were reported.

In our cohort, the MN patients (67.9 ± 10.3 years of age) were significantly older than the MCD patients (41.2 ± 18.1 years of age; $P = 0.005$), the IgAN patients (42.2 ± 20.8 years of age; $P = 0.007$), and the healthy control subjects (45.3 ± 15.5 years of age; $P = 0.0115$). All of the disease groups showed significantly lower serum albumin levels compared with healthy control group; however, no significant

difference was observed between any two disease groups. As for serum total cholesterol levels, we found that the MCD patients (422.1 ± 139.3 mg/dl) had significantly higher levels compared with the FSGS patients (324.3 ± 102.0 mg/dl; $P = 0.0461$), the IgAN patients (265.5 ± 101.0 mg/dl; $P = 0.0029$), MN patients (284.3 ± 95.0 mg/dl; $P = 0.0192$), and control subjects (206.1 ± 35.7 mg/dl; $P < 0.0001$). The FSGS patients' serum total cholesterol levels were significantly higher than those of control subjects ($P = 0.0016$). We measured C-reactive protein (CRP) in the patients with these renal diseases, because it has been reported that the serum suPAR concentration rises with nonspecific inflammation.[20–22] No significant difference in CRP was detected among the disease groups and the control group. The renal function represented by the estimated glomerular filtration rate (eGFR) was significantly higher in the control group (79.9 ± 15.8 ml/min per $1.73 \, m^2$) compared with that in the FSGS group (54.4 ± 25.6 ml/min per $1.73 \, m^2$; $P = 0.0066$). The urinary protein excretion was compared within the disease groups. The MCD patients (9138.1 ± 3874.7 mg per day or mg/gCre) excreted significantly more urinary protein than did the IgAN patients (3874.4 ± 2476.2 mg per day or mg/gCre; $P = 0.02$) or the FSGS patients (5753.2 ± 4772.3 mg per day or mg/gCre; $P = 0.04$). The amount of proteinuria in MN patients (7538.3 ± 2711.9 mg per day or mg/gCre) was also larger than in IgAN patients ($P = 0.048$).

Serum suPAR levels in primary glomerular diseases

We analyzed the unadjusted data on the serum suPAR levels according to the histological diagnosis of the glomerular diseases (Table 1). The serum suPAR levels significantly differed among the five groups, including the control group (one-way analysis of variance (ANOVA), $P < 0.0001$, effect size $f = 0.716$, power $(1 - \beta) = 0.999$). In our cohort, however, the suPAR levels in the FSGS group (3119.0 ± 1036.6 pg/ml) did not significantly differ from any other disease groups. Moreover, no significant difference was observed between any disease groups. The serum suPAR concentrations in the

Table 1 | Demographic/clinical characteristics

	All patients, $n = 69$	FSGS, $n = 38$	MCD, $n = 11$	IgAN, $n = 11$	MN, $n = 9$	Control, $n = 17$	P-value
Age (years)	52.8 ± 18.5	55.6 ± 16.3	41.2 ± 18.1	42.2 ± 20.8	$67.9 \pm 10.3^{A,B,C}$	45.3 ± 15.5	0.0007
Gender (male)	41 (59.4%)	26 (68.4%)	6 (54.5%)	5 (45.5%)	4 (44.4%)	9 (52.9%)	0.5060
Alb (mg/dl)	2.58 ± 1.00	2.56 ± 0.98	2.04 ± 0.96	3.33 ± 1.11	2.41 ± 0.37	$4.63 \pm 0.33^{D,E,F,G}$	< 0.0001
TC (mg/dl)	325.5 ± 117.9	324.3 ± 102.0^{H}	$422.1 \pm 139.3^{I,J,K,L}$	265.5 ± 101.0	284.3 ± 95.0	206.1 ± 35.7	< 0.0001
CRP (mg/dl)	0.29 ± 0.43	0.30 ± 0.47	0.23 ± 0.34	0.37 ± 0.58	0.26 ± 0.27	0.09 ± 0.13	0.4187
Steroids/immunosuppressants (yes)	19 (27.9%)	11 (29.7%)	5/6 (45.5%)	1/10 (9.1%)	2/7 (22.2%)	0 (0%)	0.0321
eGFR (ml/min per $1.73 \, m^2$)	61.9 ± 27.6	54.4 ± 25.6	76.5 ± 29.6	68.1 ± 22.0	68.4 ± 33.1	79.9 ± 15.8^{M}	0.0062
suPAR (μg/ml)	2896.8 ± 961.7	3119.0 ± 1036.6^{N}	2374.9 ± 588.8	2311.3 ± 777.1	3311.9 ± 655.3^{O}	1745.1 ± 395.4	< 0.0001
UP (mg per day or mg/g Cre)	6226.2 ± 4357.3	5753.2 ± 4772.3	$9138.1 \pm 3874.7^{P,Q}$	3874.4 ± 2476.2	7538.3 ± 2711.9^{R}	N/A	0.0211^{a}

Abbreviations: Alb, albumin; ANOVA, analysis of variance; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; FSGS, focal segmental glomerulosclerosis; HSD, honest significant difference; IgAN, IgA nephropathy; MCD, minimal change disease; MN, membranous nephropathy; NA, not available; suPAR, soluble urokinase receptor; TC, total cholesterol; UP, urinary protein.
The data are presented as the mean ± s.d. Differences among the groups were analyzed by a one-way ANOVA. The multiple comparisons for age, TC, and eGFR were performed by Tukey's HSD mean separation tests. A nonparametric Steel–Dwass test was used for Alb and suPAR. Differences between the disease groups in gender and steroids/immunosuppressants administration were determined by χ^2 tests. A: $P = 0.0048$ vs. MCD; B: $P = 0.0074$ vs. IgAN; C: $P = 0.0115$ vs. control; D: $P < 0.0001$ vs. FSGS; E: $P = 0.0002$ vs. MCD; F: $P = 0.0146$ vs. IgAN; G: $P = 0.0004$ vs. MN; H: $P = 0.0016$ vs. control; I: $P = 0.0461$ vs. FSGS; J: $P = 0.0029$ vs. IgAN; K: $P = 0.0192$ vs. MN; L: $P < 0.0001$ vs. control; M: $P = 0.0066$ vs. FSGS; N: $P < 0.0001$ vs. control; O: $P = 0.0006$ vs. control; P: $P = 0.04$ vs. FSGS; Q: $P = 0.02$ vs. IgAN; R: $P = 0.048$ vs. IgAN.
aBecause quantitative data of urinary protein for healthy controls are not available, UP in the only disease groups were analyzed by one-way ANOVA.

189

patients with FSGS and MN were significantly higher than in the healthy controls ($P < 0.0001$ and $P = 0.0006$, respectively).

Serum suPAR levels and demographic/clinical patient characteristics

Next, we assessed the association between the patients' serum suPAR levels and their demographic characteristics and clinical parameters. Although age was significantly correlated with the serum suPAR concentrations in the overall patient cohort ($R^2 = 0.1496$, $P = 0.001$, $\beta \pm$ s.e.m. $= 20.11 \pm 5.86$, effect size $\rho = 0.387$, power $(1 - \beta) = 0.930$; Figure 1), no significant difference was detected between male and female patients in the overall patient cohort (male: 2948.1 ± 884.8 pg/ml; female: 2821.5 ± 1076.9; $P = 0.60$; Figure 2). The suPAR levels in patients with nephrotic-range proteinuria (2904.6 ± 897.4 pg/ml), independent of the underlying glomerular diseases, were not significantly higher than those in patients with non-nephrotic-range proteinuria (2872.6 ± 1167.4 pg/ml, $P = 0.91$; Figure 3a). In addition, the serum suPAR levels were not correlated with urinary protein ($R^2 = 0.0020$, $P = 0.72$; Figure 3b). We did not detect significant correlations of suPAR with CRP ($R^2 = 0.053$, $P = 0.10$), serum albumin ($R^2 = 0.00048$, $P = 0.86$), or total cholesterol ($R^2 = 0.024$, $P = 0.24$). Our subgroup analysis of FSGS patients also revealed no significant correlation between suPAR and CRP ($R^2 = 0.0037$, $P = 0.82$), serum albumin ($R^2 = 0.0013$, $P = 0.83$), or total cholesterol ($R^2 = 0.0013$, $P = 0.85$).

Serum suPAR levels are inversely correlated with the eGFR

Previous studies have revealed that the serum suPAR concentration has an inverse correlation with the renal function.[13,23,24] In the current investigation, as shown in Figure 4, suPAR was correlated inversely with the eGFR ($R^2 = 0.242$, $P < 0.0001$, $\beta \pm$ s.e.m. $= -17.13 \pm 3.71$, effect size $\rho = 0.492$, power $(1 - \beta) = 0.996$) in the total patient population. Moreover, our subgroup analysis of the FSGS patient group demonstrated that there was still a significant
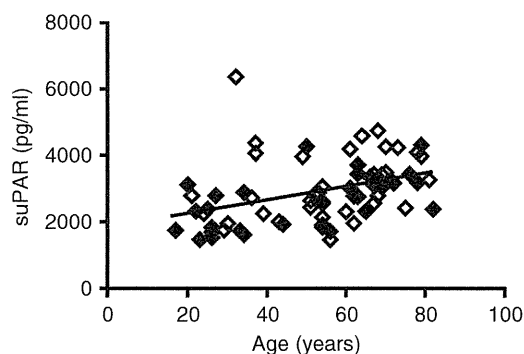
correlation between suPAR and the eGFR ($R^2 = 0.227$, $P = 0.0025$, $\beta \pm$ s.e.m. $= -19.35 \pm 5.94$, effect size $\rho = 0.476$, power $(1 - \beta) = 0.893$; Figure 4).

Serum suPAR and patient characteristics: multiple regression analysis

Next, we performed a multiple regression analysis to evaluate the association between the patients' demographic or clinical
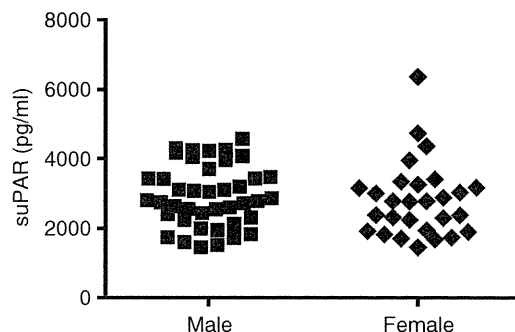
Figure 2 | Soluble urokinase receptor (suPAR) levels and gender. In the patients with primary glomerular diseases, the serum suPAR concentration did not significantly differ between male and female (unpaired *t*-test, $P = 0.60$).
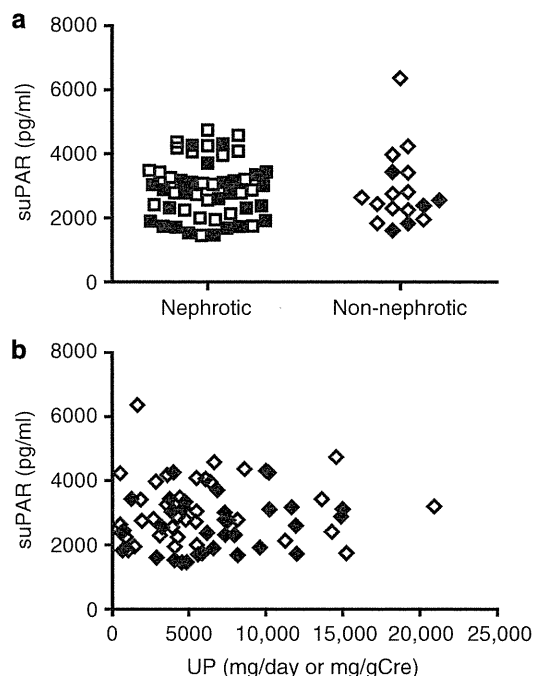
Figure 3 | Urinary protein and serum-soluble urokinase receptor (suPAR) in patients with glomerular diseases. (a) Serum suPAR levels in the patients with nephrotic-range proteinuria and non-nephrotic-range proteinuria. No significant difference was detected (unpaired *t*-test, 2904.6 ± 897.4 vs. 2872.6 ± 1167.4 pg/ml, $P = 0.91$). (b) Correlation between the urinary protein excretion (UP; mg/day or mg/gCre) and the serum suPAR levels (pg/ml). No significant correlation was observed (Pearson's correlation coefficient test, $R^2 = 0.0020$, $P = 0.72$). Open diamonds indicate focal segmental glomerulosclerosis (FSGS) patients; filled diamonds indicate patients with the other glomerular diseases.

Figure 1 | The correlation between age and the serum-soluble urokinase receptor (suPAR). In all the patients with primary glomerular diseases, the serum suPAR concentration was significantly correlated with age (Pearson's correlation coefficient test, $R^2 = 0.1496$, $P = 0.001$). Open diamonds indicate focal segmental glomerulosclerosis (FSGS) patients; filled diamonds indicate patients with the other glomerular diseases.
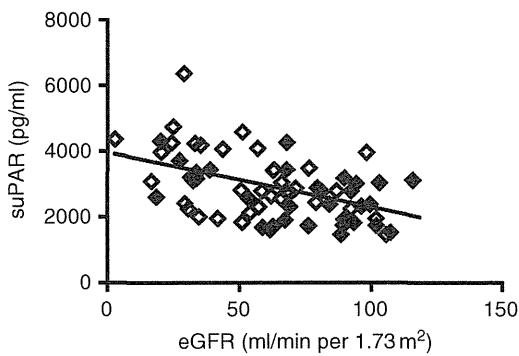
Figure 4 | The correlation between soluble urokinase receptor (suPAR) and estimated glomerular filtration rate (eGFR). Correlation between serum suPAR levels and the eGFR in all patients with primary glomerular diseases. The serum suPAR levels were significantly and inversely correlated with the eGFR (Pearson's correlation coefficient test, $R^2 = 0.242$, $P < 0.0001$). Open diamonds indicate focal segmental glomerulosclerosis (FSGS) patients; filled diamonds indicate patients with the other glomerular diseases.

Table 2 | Multiple regression analysis of the serum levels of the suPAR

	β	s.e.m.	P-value
Age (years)	0.230	0.230	0.068
Gender (male)	− 0.032	0.111	0.773
Urinary protein (mg per day or mg/gCre)	0.067	0.119	0.580
Serum creatinine (mg/dl)	0.226	0.226	0.047
Disease (FSGS)	0.214	0.124	0.090
Disease (IgAN)	− 0.213	0.135	0.121
Disease (MCD)	− 0.123	0.134	0.365
Disease (MN)	0.121	0.102	0.241
Steroids/immunosuppressants administration (yes)	0.208	0.115	0.076

Abbreviations: FSGS, focal segmental glomerulosclerosis; IgAN, IgA nephropathy; MCD, minimal change disease; MN, membranous nephropathy; suPAR, soluble urokinase receptor.

parameters and their suPAR levels. Because the eGFR depends upon the age, gender, and serum creatinine concentration, we used serum creatinine instead of eGFR as a predictor variable. The other predictor variables were age, gender, urinary protein, disease groups, and steroid/immuno-suppressant administration. The analysis ($R^2 = 0.323$, $P = 0.002$) demonstrated that serum creatinine ($\beta \pm$ s.e.m. = 0.226 ± 0.226, $P = 0.0472$) was significantly correlated with the serum suPAR (Table 2). The effect size f^2 and power $(1 − \beta)$ of the analysis were 0.300 and 0.918, respectively. These findings suggest that the renal function significantly affects the suPAR levels, which is consistent with the results from the above-described univariate analyses.

Serum suPAR levels in patients with normal renal function

Because renal impairment is closely associated with the suPAR levels, we decided to thereafter limit the objectives to patients with normal renal function. In accordance with the definition of chronic kidney disease, we excluded patients whose eGFR was below 60 ml/min per 1.73 m^2. For this
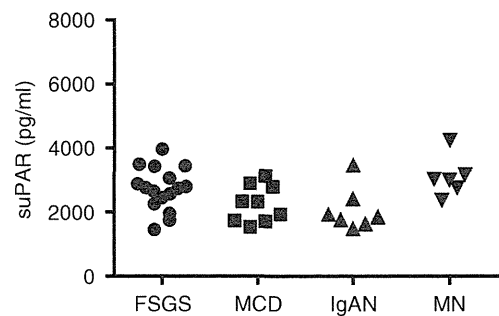
suPAR	FSGS	MCD	IgAN	MN
Mean	2723.0	2260.6	2055.0	3110.2
s.d.	671.2	577.2	678.1	630.9

Figure 5 | Serum levels of the soluble urokinase receptor (suPAR) in patients with primary glomerular disease whose estimated glomerular filtration rate (eGFR) was 60 ml/min per 1.73 m^2 or higher. Patients whose eGFR was 60 ml/min per 1.73 m^2 or higher were distinguished and analyzed for their serum suPAR levels. There was significant difference in suPAR levels among the disease groups (one-way analysis of variance (ANOVA), $R^2 = 0.254$, $P = 0.018$, effect size $f = 0.584$, power $(1 − \beta) = 0.824$); however, no significant difference was detected in a multiple comparison (Steel–Dwass test).

subgroup, the difference in suPAR levels between any disease groups was not significant by multiple comparisons (Figure 5).

Diagnostic value of suPAR: differentiating FSGS from the other glomerular diseases

Because primary FSGS and MCD often exhibit similar clinical presentations and because there are difficulties in differentiating these two diseases even with a renal biopsy, a potent diagnostic biomarker has long been awaited. In our overall cohort, which included patients with low GFR, the distribution of serum suPAR levels in the FSGS patients demonstrated that there was apparently a subpopulation that exhibited higher suPAR levels than did the other disease groups, although the difference in suPAR between the disease groups did not reach statistical significance (Table 1). This finding motivated us to test the diagnostic value of suPAR for differentiating primary FSGS from MCD in patients without renal dysfunction. We performed a receiver operating characteristic (ROC) curve analysis on the FSGS patients ($n = 16$) and the MCD patients ($n = 9$) without renal dysfunction. By identifying the point at which the difference between sensitivity and 1-specificity is maximal, we determined that the optimal cutoff value should be 2442.5 pg/ml of the suPAR concentration for these FSGS or MCD patients (Figure 6a; solid line). For this cutoff value, the sensitivity and specificity of this test were 0.750 and 0.666, respectively. The positive likelihood ratio (LR) was 2.25, which means that the suPAR levels higher than 2442.5 pg/ml do not usefully increase the probability that FSGS exists (the post-test odds). Moreover, the negative LR was 0.625, suggesting that the suPAR levels lower than 2442.5 pg/ml do not sufficiently change the probability that a patient does not have FSGS. No
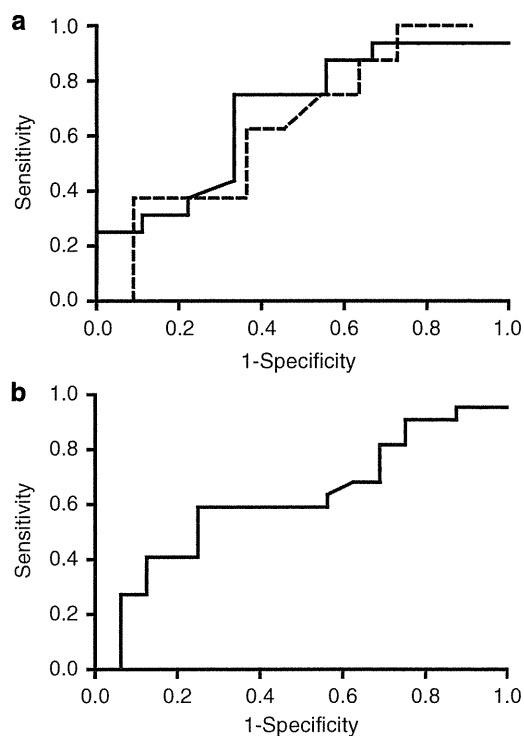
Figure 6 | Receiver operating characteristic (ROC) analysis of the serum soluble urokinase receptor (suPAR) in the patients with focal segmental glomerulosclerosis (FSGS). (a) ROC analysis in the patients with FSGS or minimal change disease (MCD). Solid line, ROC curve for patients with FSGS or MCD whose estimated glomerular filtration rate (eGFR) was 60 ml/min per 1.73 m^2 or higher. The area under the ROC curve (AUC-ROC) was 0.684 ± 0.114 (95% confidence interval (CI): 0.461–0.907, *P* = 0.13); dotted line, ROC curve for the nephrotic patients with FSGS or MCD whose eGFR was 60 ml/min per 1.73 m^2 or higher. The AUC-ROC was 0.642 ± 0.130 (95% CI: 0.388–0.896, *P* = 0.30). **(b)** ROC analysis in the patients with FSGS or the other glomerular diseases whose eGFR was 60 ml/min per 1.73 m^2 or higher. The AUC-ROC was 0.621 ± 0.093 (95% CI: 0.438–0.803, *P* = 0.21).

significant difference between the FSGS and MCD patients was detected regarding the probability to exhibit suPAR levels higher than the cutoff value (*P* = 0.06). The area under the ROC curve (AUC-ROC) for this patient population was 0.684 ± 0.114 (95% confidence interval (CI): 0.461–0.907, *P* = 0.13). These data suggest that the suPAR level is not useful to differentiate FSGS from MCD. By contrast, when we set 3000 pg/ml as a cutoff value, as Wei *et al.*[10,12] did in the previous studies, the sensitivity and specificity of this test were 0.313 and 0.889, respectively, suggesting that this cutoff value would yield an extremely low sensitivity.

Both primary FSGS and MCD are typically characterized by nephrotic syndrome with an abrupt onset; however, the prognosis and the response to therapy differ from each other. When we limited the objectives to nephrotic (serum albumin < 3.0 g/dl and urinary protein ≥ 3.5 g per day or g/g Cre) FSGS (*n* = 11) or MCD (*n* = 8) patients, the ROC curve analysis suggested that the optimal cutoff value under this condition should be 1748.75 pg/ml (Figure 6a; dotted line).

The sensitivity, specificity, positive LR, and negative LR were 0.909, 0.375, 1.45, and 0.24, respectively. The AUC-ROC for the nephrotic patient group was limited to 0.642 ± 0.130 (95% CI: 0.388–0.896, *P* = 0.30). When we tested whether the serum suPAR levels are useful for differentiation between FSGS (*n* = 16) and the other glomerular diseases (*n* = 22), the optimal cutoff value was 2442.5 pg/ml. The sensitivity, specificity, positive LR, and negative LR were 0.750, 0.591, 1.83, and 0.423, respectively. The AUC-ROC was 0.621 ± 0.093 (95% CI: 0.438–0.803, *P* = 0.21), suggesting that serum suPAR levels are not useful to discriminate FSGS from the other glomerular diseases (Figure 6b). Taken together, these data suggest that the serum suPAR concentration is not a potent diagnostic marker for clinical use.

suPAR levels in the patients taking steroids and/or immunosuppressants

Steroids and immunosuppressants are often used to treat primary glomerular diseases, including FSGS. To evaluate the association between the use of steroids/immunosuppressants and the suPAR levels, we compared the serum suPAR levels in patients with and without those medications. As shown in Figure 7a, all the patients with primary glomerular diseases who took steroids and/or immunosuppressants (IS(+)) tended to have lower serum suPAR levels; however, this difference did not reach statistical significance (IS(−) vs. IS(+) = 3039 ± 1004 vs. 2559 ± 789.2 pg/ml, *P* = 0.06, effect size *d* = 0.532, power (1 − β) = 0.489). By contrast, the FSGS patients without renal dysfunction who took steroids and/or immunosuppressants had significantly lower levels of suPAR compared with the FSGS patients not taking these medications (Figure 7b: IS(−) vs. IS(+) = 3076.88 ± 498.52 vs. 2170.00 ± 533.68 pg/ml, *P* = 0.009, effect size *d* = 1.756, power (1 − β) = 0.842). However, urinary protein excretion, which is associated with disease activity, did not differ significantly between the untreated and treated FSGS groups (IS(−) vs. IS(+) = 5453.8 ± 3261.9 vs. 4986.5 ± 6015.8 mg per day or mg/gCre, *P* = 0.365). Furthermore, the use of steroids/immunosuppressants was not a significant predictive variable for the suPAR levels in the multiple regression equation as shown above (Table 2).

The suPAR levels of MCD patients with and without steroids/immunosuppressants were 2488.8 ± 628.9 and 1975.3 ± 407.0 pg/ml, respectively, and the difference was not significant (*P* = 0.270), whereas urinary protein excretion did not differ between the two MCD groups (IS(−) vs. IS(+) = 7857.8 ± 3257.9 vs. 10495.6 ± 4308.9 mg per day or mg/gCre, *P* = 0.5403). Among MN patients, only one patient was treated with steroid. The patient's serum suPAR concentration was 3017.5 pg/ml, which was almost the same level as MN patients without steroids/immunosuppressants (3128.8 ± 703.6 pg/ml). Urinary protein of the MN patient who took steroid was 7360 mg per day, which was largely the same level as the untreated MN patients' urinary protein (6670.9 ± 3207.0 mg per day or mg/gCre). In our cohort, none of IgAN patients with normal renal function took
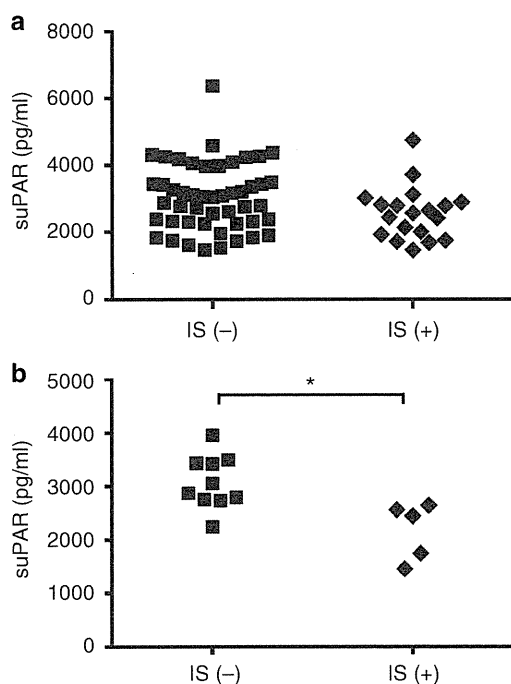
Figure 7 | Treatment with steroids/immunosuppressants and the serum soluble urokinase receptor (suPAR) levels. (a) Comparison of the suPAR levels between glomerular disease patients with normal glomerular function who were treated with steroids or immunosuppressants (IS(+)) and those who were not (IS(−)). No significant difference was detected (unpaired t-test, 2559.3 ± 789.2 vs. 3047.0 ± 994.9 pg/ml, $P = 0.06$). (b) Comparison of the suPAR levels between the focal segmental glomerulosclerosis (FSGS) patients with normal renal function who were treated with steroids or immunosuppressants (IS(+)) and those who were not (IS(−)). The IS(+) patients presented significantly lower suPAR levels (Mann–Whitney U-test, 2170.0 ± 533.7 vs. 3076.9 ± 498.5 pg/ml, *$P = 0.009$).

steroids or immunosuppressants. Taken together, although treatment with steroids and/or immunosuppressants was associated with lower suPAR levels in the FSGS group, it is still inconclusive whether lower suPAR levels are associated with disease activity. The relationships between suPAR, steroid/immunosuppressants, and disease activities in the other disease groups are also obscure. A longitudinal study would be necessary to draw the conclusion.

High suPAR levels in ANCA–associated glomerulonephritis

As a separate cohort, we evaluated the serum suPAR concentrations in the five patients with antineutrophil cytoplasmic antibody (ANCA)–associated glomerulonephritis (ANCA-GN). Their characteristics are as follows (average ± s.d.): age, 67.8 ± 7.9 years; gender, male/female = 1/4; eGFR, 13.0 ± 5.2 ml/min per 1.73 m²; urinary protein, 912 ± 140 mg per day or mg/gCre; serum albumin, 3.01 ± 0.78 g/dl; serum total cholesterol, 189.4 ± 65.3 mg/dl; and CRP, 5.14 ± 5.6 mg/dl. The suPAR concentration in this patient group was 6791.3 ± 1513.0 pg/ml, which was substantially higher than that in the patients with the primary glomerular diseases

described above. When we compared this cohort with the subgroup of non-ANCA-GN patients matched for age and eGFR (3 FSGS, 1 MCD, and 1 MN; age, 61.4 ± 16.3 years; eGFR, 16.5 ± 8.1 ml/min per 1.73 m²; and suPAR, 3727.5 ± 818.2 pg/ml), the suPAR levels in the ANCA-GN patients were significantly higher than in the non-ANCA-GN patients (data not shown, Mann–Whitney U-test, $P = 0.01$, effect size $d = 2.519$, power $(1 − \beta) = 0.935$). Although the sample size was small, it suggested that inflammation might affect the suPAR concentration. Given the inverse correlation between suPAR and the eGFR, these data suggest that the high suPAR concentration in ANCA-GN patients might be attributable to impaired renal function and inflammation.

DISCUSSION

In the current study, we performed a multicenter cross-sectional study of suPAR levels in Japanese patients with glomerular diseases to explore the usefulness of suPAR as a diagnostic marker. The first finding in this study was that the suPAR levels were inversely correlated with the eGFR. Previous investigations have demonstrated an inverse correlation between suPAR and eGFR;[13,23,24] thus, the present finding is consistent with those data. Wei et al.[12] also demonstrated an inverse correlation between suPAR and eGFR in their study of two large cohorts. Because the molecular weight of the major fragment of suPAR is 22 kDa, which should be small enough to be filtered through the glomerular filtration barrier, it seems reasonable that suPAR levels are inversely correlated with renal function. Although a kinetic study will be necessary to confirm this speculation, it is likely that suPAR accumulates in patients with renal impairment. On the basis of this finding, we performed an analysis on the patients whose eGFR levels were 60 ml/min per 1.73 m² or more. The second finding was that the suPAR levels did not have diagnostic value for differentiating FSGS from MCD or the other glomerular diseases. Moreover, we evaluated the suPAR levels only in nephrotic patients with FSGS and MCD, and the result was equivalent. Because primary FSGS often exhibits a clinical presentation similar to MCD, it is sometimes not easy to distinguish FSGS from MCD, even with a renal biopsy. Therefore, a reliable diagnostic biomarker would not only be useful to distinguish these two diseases but may also elucidate the pathogenesis of these disorders causing nephrotic syndrome. Garin et al.[25] reported a significant increase in the urinary excretion of CD80 (also known as B7-1) from MCD patients, but not from FSGS patients. Thus, the development of a biomarker that distinguishes FSGS from MCD has been at the center of attention in this field. However, our data suggest that serum suPAR cannot serve in this role.

The third finding was that FSGS patients without renal impairment who took steroids/immunosuppressants present significantly lower levels of suPAR. We cannot exclude the possibility that suPAR is associated with the pathogenesis; however, the association is still inconclusive because of the small sample size and inconsistent result with multiple

regression analysis. Moreover, urinary protein excretion, a clinical parameter for disease activity, was not different between untreated and treated FSGS patients in our cohort. Given that suPAR levels are increased along with nonspecific inflammation, a decrease in suPAR may be simply associated with direct effects of these medications. A longitudinal study of a larger cohort will be necessary to clarify the association between suPAR levels and the clinical outcomes of FSGS.

The fourth finding was that the ANCA-GN patients presented remarkably higher levels of serum suPAR than were observed in patients with other primary glomerular diseases. Several factors should be considered for ANCA-GN. The first factor is renal function, as the average eGFR in our ANCA-GN patients was 13.0 ml/min per 1.73 m^2. Given the previous findings and our data in this study, impaired renal function may cause increased suPAR levels. The second factor that potentially affected the suPAR levels was age. Five patients with ANCA-GN were older than the patients in the other disease groups. Given our data demonstrating the correlation of age and suPAR, aging may have a causal role in suPAR accumulation. Further studies will be necessary to define the precise relationship between age and suPAR. The other factor that may potentially affect the suPAR level is inflammation. Previous studies have suggested an association between increased suPAR levels and nonspecific inflammation,[20–22] and the CRP levels for the five patients evaluated in this study were 5.14 mg/dl, on average. The comparison of the suPAR levels between the ANCA-GN patients and the age- and eGFR-matched subgroup from our cohort revealed that the suPAR levels in the ANCA-GN patients were still significantly higher, suggesting that inflammation might have a role.

Several limitations might affect the results obtained in this study. First, although most of the analyses in the current investigation had sufficient power, the sample size of this study was still relatively small. A pathologic diversity of glomerular lesions in FSGS is evident; however, the relatively small power did not allow us to analyze the suPAR levels in each FSGS subtype. A detailed classification of FSGS patients (requiring a larger number of subjects) might give us more precise information on the association of suPAR and the development of FSGS. Second, the histological diagnosis was performed by the pathologists at each facility and was not standardized. Third, we could not evaluate the relationship between the change in the suPAR levels and the clinical outcomes because of the cross-sectional nature of this investigation. Longitudinal follow-up studies might give us a new insight into the pathological roles of suPAR. Finally, the ELISA system we used measures only the complete form of uPAR, whereas some splicing forms do exist, and there might be an FSGS-specific form of suPAR.

In conclusion, this study suggests that the serum suPAR level is affected by age, renal function, or inflammation. Furthermore, in this investigation, serum suPAR levels had little value for differentiating FSGS from the other glomerular diseases, especially MCD.

MATERIALS AND METHODS

Patient population

We conducted a multicenter cross-sectional study for serum suPAR levels in Japanese patients with primary glomerular diseases who underwent renal biopsy in the participating nephrology divisions. This study was planned and conducted by the Japanese Refractory Nephrotic Syndrome Study Group of the Ministry of Health, Labor and Welfare of Japan. We studied 70 patients with biopsy-proven primary glomerular diseases from eight different hospitals in Japan. All patients had undergone renal biopsy, and pathologists in each hospital performed the pathohistological diagnoses. After one sample collected from a patient with MCD was excluded as an outlier for the suPAR concentration based on the Mahalanobis distance, we studied 69 patients: 38 with FSGS, 11 with MCD, 11 with IgAN, and 9 with MN. Patients with a clinically identifiable cause of these diseases had been excluded. As for FSGS, patients with potential causes of secondary FSGS (i.e. obesity, family history of FSGS, drug, viral infection, and structural maladaptation) were not included. We also measured the serum suPAR concentrations in 17 healthy volunteers. In addition, the serum suPAR levels in five patients with ANCA-GN were evaluated. For one-way ANOVA analysis among four groups for glomerular diseases and a group for the healthy control, 80 samples were required when we set the effect size at 0.40, the α-value at 0.05, and the power $(1 - \beta)$ at 0.8. The study protocol was approved by the Institutional Review Boards of the University of Tokyo and of each participating hospital. Informed consent was obtained from each participant.

The recorded clinical parameters included the clinical diagnosis; age; gender; urine protein/creatinine ratio or 24-h urinary protein; steroid and immunosuppressant therapy status; and serum levels of creatinine, albumin, and total cholesterol. The eGFR was calculated using the Japanese eGFR equation.[26] Histological information was also derived from each patient's medical record. The patients were categorized according to their histological diagnoses.

Serum suPAR measurement

Serum samples were collected from the patients on the day of renal biopsy. The sera were separated by centrifugation and frozen at − 80 °C until the measurement. During the investigation, refreezing the thawed samples was avoided. We performed duplicate measurements of the serum suPAR levels, using the Quantikine Human suPAR Immunoassay (R&D Systems, Minneapolis, MN), which is a solid-phase sandwich enzyme-linked immunosorbent assay kit, according to the manufacturer's protocol. This enzyme-linked immunosorbent assay kit has been used in most of the previous studies[10,12,13,17,18] of suPAR levels and FSGS, including the original investigations by Wei *et al.*[10,12]

Statistical analyses

The data are expressed as the mean ± s.d., for the continuous variables, whereas standard errors of the mean are shown for AUC-ROC and regression coefficients. We evaluated the differences in each biochemical parameter (including suPAR) among the glomerular diseases by a one-way ANOVA followed by multiple-comparison analyses. Each multiple-comparison analysis was performed with Tukey's HSD (honest significant difference) mean separation test (parametic) or the Steel–Dwass test (nonparametric), depending on the normality of the data distribution determined by the D'Agostino–Pearson omnibus normality test. Correlations between the suPAR level and the demographic or clinical parameters

were evaluated using Pearson's correlation coefficient test. For the comparison between the patients with steroids/immunosuppressants and those without, the unpaired *t*-test (for all patients) or the nonparametric Mann–Whitney *U*-test (for the FSGS group and the MCD group) was used. For the comparison between the ANCA-GN patient group and the subgroup matched for age and eGFR, the nonparametric Mann–Whitney *U*-test was used. We used the χ^2 test to evaluate the differences in positive ratios at the selected cutoff values between FSGS and MCD. Statistical analyses were performed using the JMP 9.0 statistical software (SAS Institute, Cary, NC) and GraphPad Prism version 5.04 software (GraphPad Software, San Diego, CA). Power analyses were performed using the G*Power 3 software (Heinrich-Heüine Universität Düsseldorf, Düsseldorf, Germany). *P*-values <0.05 were considered significant.

DISCLOSURE
All the authors declared no competing interests.

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ORIGINAL ARTICLE

Significance of combined cyclosporine–prednisolone therapy and cyclosporine blood concentration monitoring for idiopathic membranous nephropathy with steroid-resistant nephrotic syndrome: a randomized controlled multicenter trial

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Abstract

Background Combined treatment with cyclosporine microemulsion preconcentrate (CyA MEPC) and steroids has been widely used for idiopathic membranous nephropathy (IMN) associated with steroid-resistant nephrotic syndrome (SRNS). Recent studies have shown that once-a-day and preprandial administration of CyA MEPC is more advantageous than the conventional twice-a-day administration in achieving the target blood CyA concentration at 2 h post

The other authors on behalf of the Refractory Nephrotic Syndrome Study Group are listed in the Appendix.

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dose (C2). We designed a randomized trial to compare these administrations.
Methods IMN patients with SRNS (age 16–75 years) were divided prospectively and randomly into 2 groups. In group 1 ($n = 23$), 2–3 mg/kg body weight (BW) CyA MEPC was given orally once a day before breakfast. In group 2 ($n = 25$), 1.5 mg/kg BW CyA MEPC was given twice a day before meals. CyA + prednisolone was continued for 48 weeks.
Results Group 1 showed a significantly higher cumulative complete remission (CR) rate ($p = 0.0282$), but not when incomplete remission 1 (ICR1; urine protein 0.3–1.0 g/day) was added ($p = 0.314$). Because a C2 of 600 ng/mL was determined as the best cut-off point, groups 1 and 2 were

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