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 ($n = 32$, $\rho = 0.576$, $p = 0.001$,

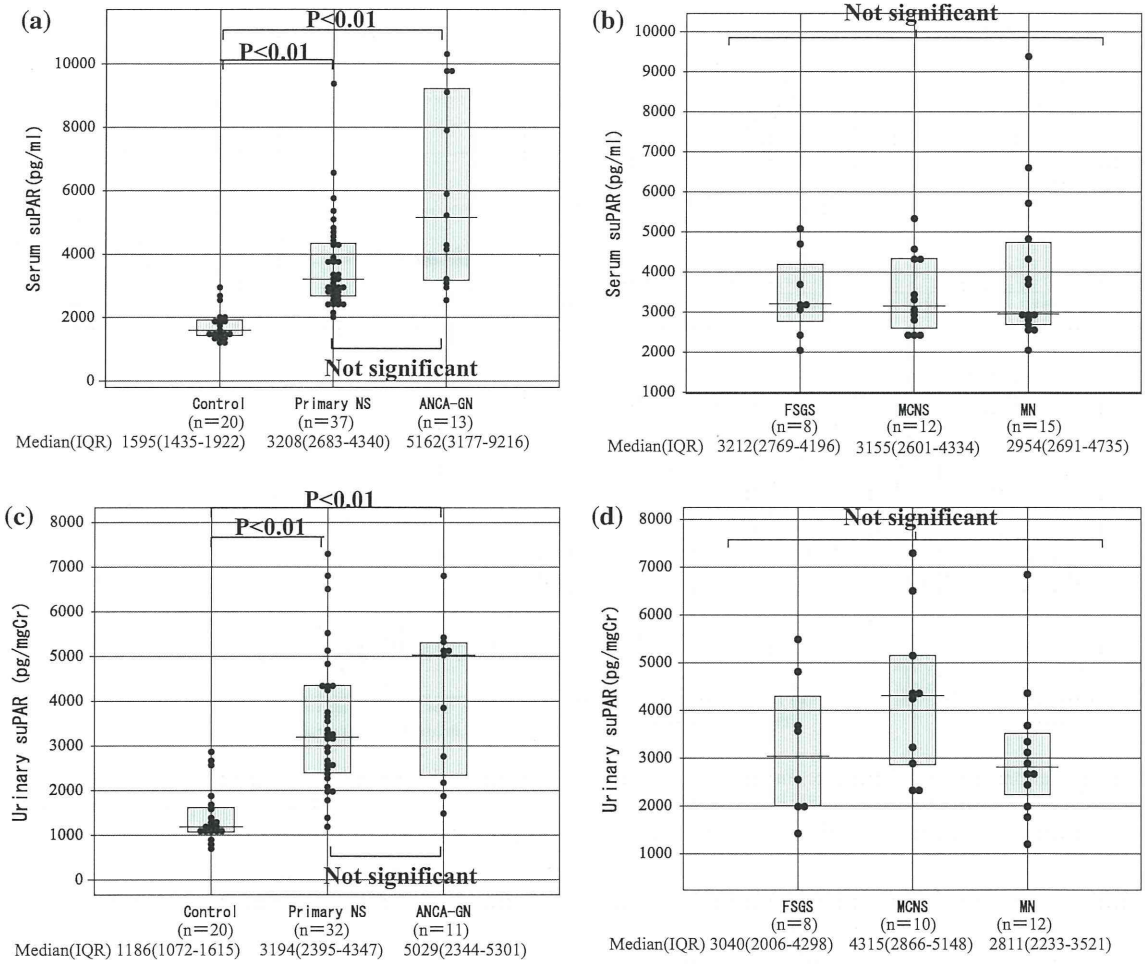

Fig. 1 Comparison of serum and urinary suPAR levels before therapy **a** Serum suPAR levels before therapy were significantly higher in patients with primary NS or ANCA-GN than in normal controls ($p < 0.01$). On the other hand, no significant differences were noted in pretreatment serum suPAR levels 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, $p = 0.499$,

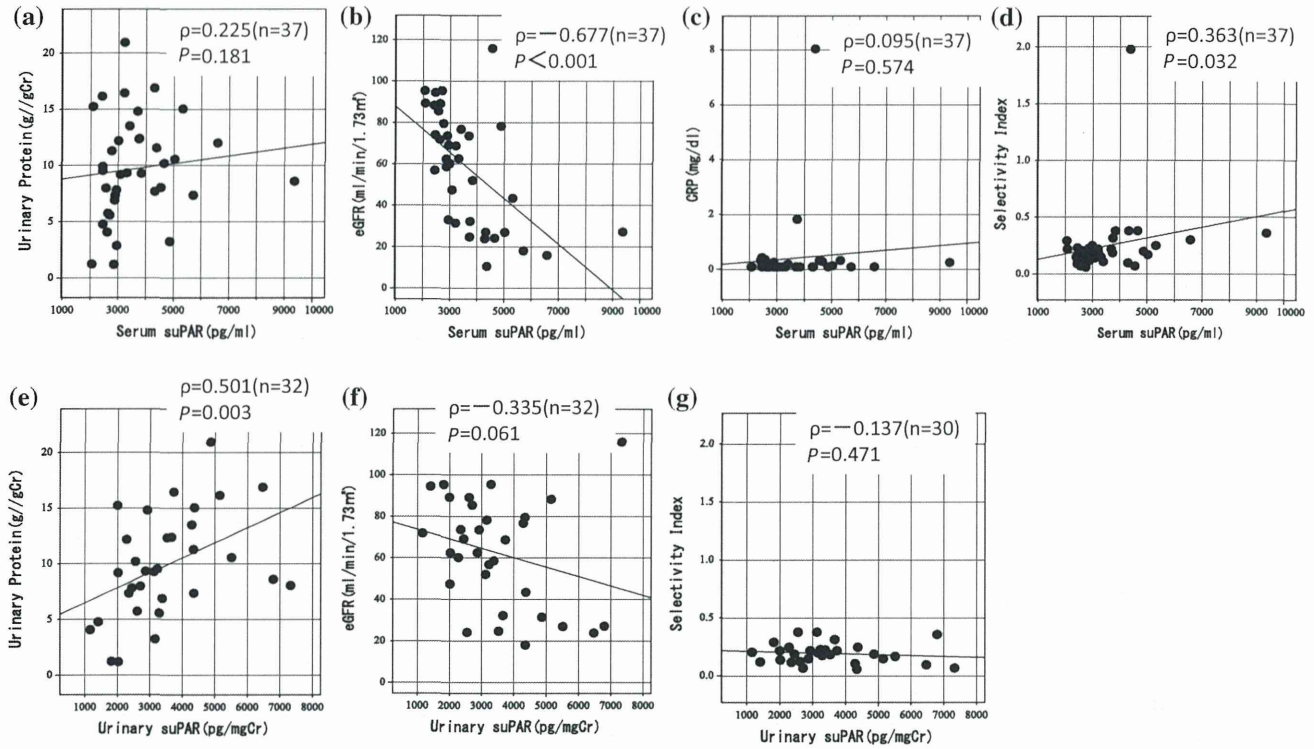

Fig. 2 The correlations between serum or urinary suPAR levels before therapy and the clinical parameters in primary NS. **a** Serum suPAR and urinary protein, **b** serum suPAR and eGFR, **c** serum suPAR and CRP, **d** serum suPAR and selectivity index, **e** urinary suPAR and urinary protein, **f**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

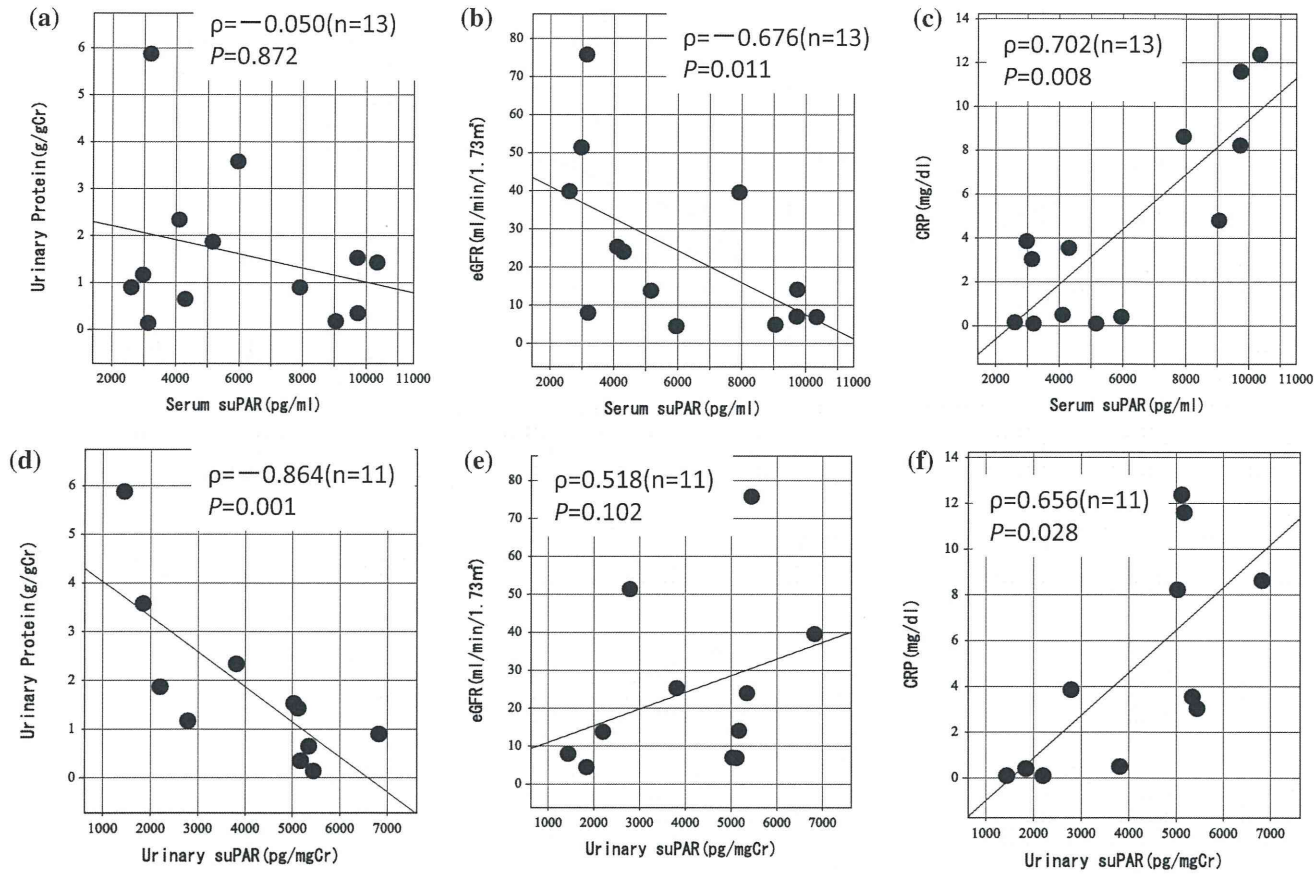

Fig. 3 The correlations between serum or urinary suPAR levels before therapy and the clinical parameters in ANCA-GN. **a** Serum suPAR and urinary protein, **b** serum suPAR and eGFR, **c** serum suPAR and CRP, **d** urinary suPAR and urinary protein, **e** urinary suPAR and eGFR, **f** urinary suPAR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

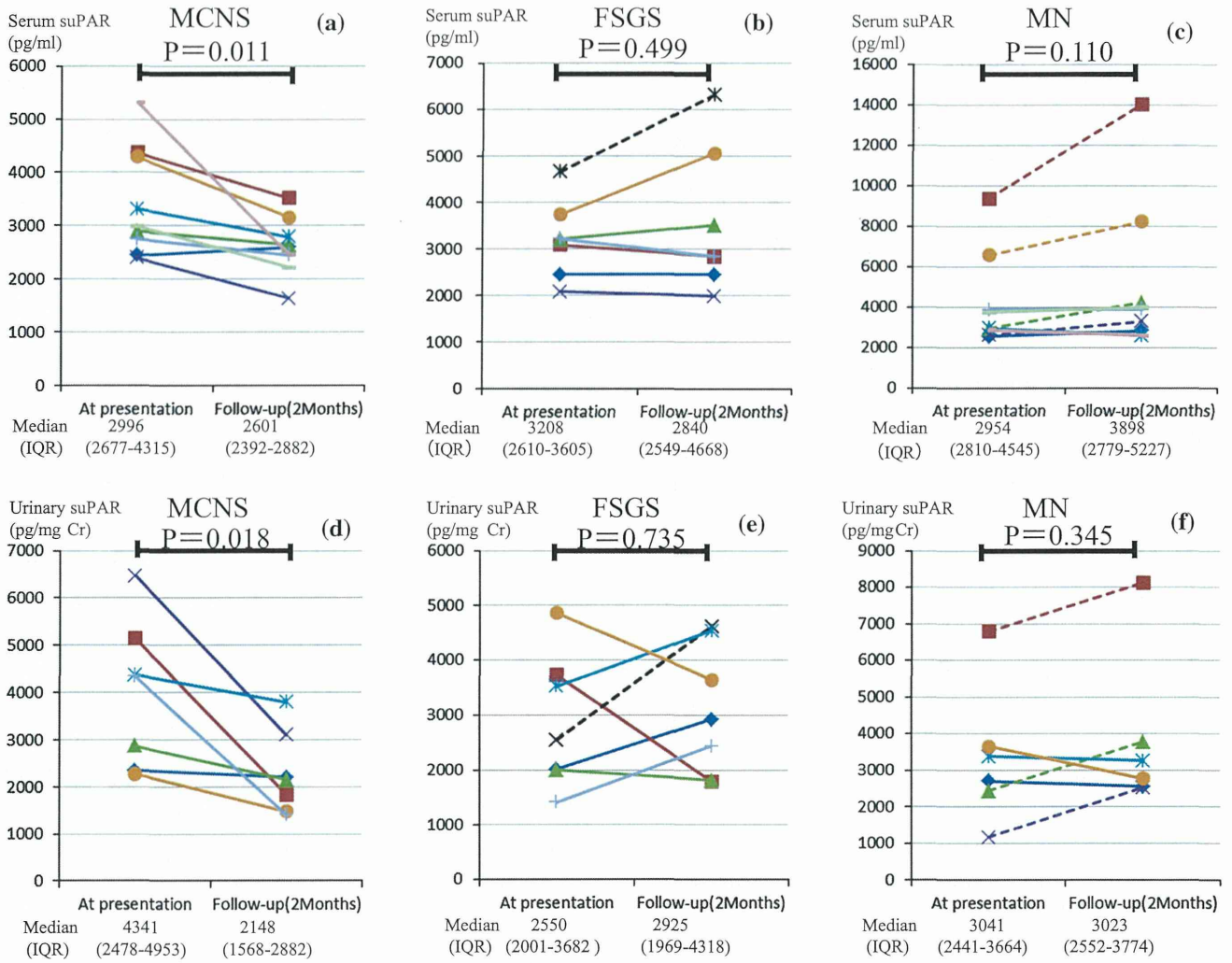

Fig. 4 The changes of serum and urinary suPAR levels after therapy among the disease types of primary NS *Solid lines* are non-refractory cases. *Dotted lines* are the refractory cases. In patients with MCNS (**a**) (**d**), serum and urinary suPAR levels significantly decreased. In patients with FSGS (**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

Table 2 Receiver operating characteristic (ROC) analysis of clinical parameters as predictors of intractable NS or FSGS

Clinical parameters	AUC-ROC	Cut-off value	Sensitivity (=specificity)	*p*	*n*
The diagnostic performance of differentiating intractable NS from non-intractable NS					
2M s-suPAR	0.913	3,373 (pg/mL)	0.762	0.002	27
Δ2M s-suPAR	0.881	−189 (pg/mL)	0.833	0.005	27
2M u-suPAR	0.776			0.066	22
Δ2M u-suPAR	0.906	−127 (pg/mgCr)	0.800	0.007	22
2M UP	0.968	0.97 (g/gCr)	0.952	0.001	27
Δ2M UP	0.833	−6.97 (g/gCr)	0.762	0.014	27
2M eGFR	0.730			0.091	27
Δ2M eGFR	0.579			0.560	27
The diagnostic performance of differentiating FSGS from MCNS					
2Ms-suPAR	0.698			0.186	16
Δ2M s-suPAR	0.905	−268 (pg/mL)	0.857	0.007	16
2M u-suPAR	0.694			0.225	14
Δ2M u-suPAR	0.816	−718 (pg/mgCr)	0.714	0.048	14
2M UP	0.675			0.244	16
Δ2M UP	0.508			0.958	16
2M eGFR	0.587			0.560	16
Δ2M eGFR	0.651			0.315	16

The cut-off value is at the point where the specificity equal to the sensitivity

NS nephrotic syndrome, *FSGS* focal segmental glomerulosclerosis, *MCNS* minimal change nephrotic syndrome, *s-* serum, *u-* urinary; *2M* at 2 months after therapy, *Δ2M* change during 2 months after therapy, *UP* urinary protein, *AUC-ROC* the area under the curve of receiver operating characteristic analysis

factors that influence vital and renal prognoses in a study on Japanese ANCA-GN, and these were found to be important evaluation items to judge the clinical severity of ANCA-GN. Based on these findings, we assumed that s-suPAR was associated with the clinical severity of ANCA-GN, suggesting that s-suPAR is a useful clinical severity marker of ANCA-GN. In addition, a positive correlation was noted between pretreatment s-suPAR and crescent formation in this study. Baraldi et al. [19] detected the strong expression of β3 integrin on crescent cells, podocytes, and Bowman's capsular epithelial cells in RPGN patients, and suggested the involvement of β3 integrin in the mechanism underlying crescent formation. Thus, it was also assumed that s-suPAR activated β3 integrin on podocytes and Bowman's capsular epithelial cells, and this process was part of the mechanism underlying crescent formation in ANCA-GN as well as crescentic FSGS [4].

Regarding s-suPAR as a clinical marker, we also investigated the time-course of s-suPAR over 2 months of immunosuppressive therapy because s-suPAR over 3,000 pg/mL was previously shown to activate podocyte β3 integrin [1]. Although intractable and non-intractable cases could not be differentiated based on s-suPAR over 3,000 pg/mL before immunosuppressive therapy, which is consistent with previous findings [7], 3,373 pg/mL or higher at 2 months may be used for a marker to judge responses to treatments. These changes in s-suPAR after therapy were not significantly influenced by changes in eGFR or CRP in this study. Moreover, 2M s-suPAR was positively correlated with 2M UP and a cut-off value of 2M s-suPAR to differentiate intractable cases was close to the s-suPAR level (3,000 pg/mL) in the first study [1]. Reiser et al. may have observed this phenomenon in primary FSGS cases treated with immunosuppressive drugs.

In our study, differentiating between FSGS and MCNS was possible based on changes in s-suPAR over 2 months of immunosuppressive therapy. Huang et al. [4] compared s-suPAR before immunosuppressive therapy with that after 78 weeks of therapy on average in primary FSGS patients, and observed a significant decrease in complete remission cases, no change in incomplete remission cases, and a significant elevation in ineffective cases. These findings revealed changes in s-suPAR that corresponded to the responses to treatments. Similar to our results, they could not predict therapeutic responses in FSGS patients based on s-suPAR before immunosuppressive therapy [4]. On the other hand, no significant change was noted in s-suPAR over the 2-month period in this study. Considering that the follow-up period was markedly longer (78 weeks on average) in their study than in ours (8 weeks), the 2-month period may have been too short to observe a reduction in

s-suPAR in FSGS, even for patients showing favorable responses to treatments. On the other hand, s-suPAR rapidly decreased within the 2-month period in MCNS, suggesting that the 2-month s-suPAR reduction rate serves as an index to differentiate MCNS.

We also investigated u-suPAR as a clinical marker in primary NS. Pretreatment u-suPAR was significantly higher in the primary NS and ANCA-GN groups. In contrast to recent findings [5], no significant differences were noted between the primary NS and ANCA-GN groups, or among the disease types of primary NS (FSGS, MCNS, and MN). Since pretreatment u-suPAR positively correlated with UP in all primary NS, similar to previous findings [6], we suspected that u-suPAR may be the only initial indication of UP. However, u-suPAR only decreased after treatments were received by non-intractable NS and MCNS patients, but not by FSGS or MN patients. Thus, we assessed whether changes in u-suPAR could be used to differentiate non-intractable NS from intractable NS or MCNS from FSGS. A ROC analysis revealed that Δ2M u-suPAR was a useful marker for differentiating non-intractable NS from intractable NS or MCNS from FSGS. Huang et al. [5] also reported higher u-suPAR levels in the cellular variant and significant decreases in u-suPAR, even in primary FSGS patients with complete remission. In our study, tip lesions were detected in the majority of FSGS patients. Hence, further investigations are needed to resolve the issue of u-suPAR in MCNS and the different pathological lesions of FSGS such as tip lesions and the cellular variant.

In conclusion, s- or u-suPAR may be useful as an index of treatment responses by patients with primary NS for the differentiation of MCNS from FSGS, but not in pretreatment patients. In addition, our study revealed that s- and u-suPAR were associated with the long-term therapeutic responses of all primary NS patients including those with MCNS, FSGS, MN and MPGN. S- and u-suPAR were significantly decreased in MCNS after therapy and could be used to differentiate MCNS from FSGS. In addition, s-, but not u-suPAR levels before therapy may be useful for judging the clinical severity of and crescent formation in ANCA-GN. The ELISA system used in this study only measured the complete form of suPAR; however, several splicing forms and different glycosylation forms are known to exist. Thus, it is also possible that the molecular size or glycosylation of suPAR differs among these diseases, and differences in physiological activity due to these variations in suPAR may lead to differences in the renal histological phenotype. These issues need to be investigated in future studies.

Acknowledgments The authors gratefully acknowledge the help and assistance of their colleagues at the Division of Nephrology. This study was supported, in part, by a Grant-in-Aid for Progressive Renal Disease Research from the Ministry of Health, Labour, and Welfare of Japan and a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (HY, (C) No. 25461237; (B) No. 24406029, No. 25305028).

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

References

1. Wei C, El Hindi S, Li J, Fornoni A, Goes N, Sageshima J, et al. Circulating urokinase receptor as a cause of focal segmental glomerulosclerosis. Nat Med. 2011;17:952–60.
2. Wei C, Moller CC, Altintas MM, Li J, Schwarz K, Zacchigna S, et al. Modification of kidney barrier function by the urokinase receptor. Nat Med. 2008;14:55–63.
3. Wei C, Trachtman H, Li J, Dong C, Friedman AL, Gassman JJ, et al. Circulating suPAR in two cohorts of primary FSGS. J Am Soc Nephrol. 2012;23:2051–9.
4. Huang J, Liu G, Zhang YM, Cui Z, Wang F, Liu XJ, et al. Plasma soluble urokinase receptor levels are increased but do not distinguish primary from secondary focal segmental glomerulosclerosis. Kidney Int. 2013;84:366–72.
5. Huang J, Liu G, Zhang YM, Cui Z, Wang F, Liu XJ, et al. Urinary soluble urokinase receptor levels are elevated and pathogenic in patients with primary focal segmental glomerulosclerosis. BMC Med. 2014;12:81. doi:10.1186/1741-7015-12-81.
6. Sinha A, Bajpai J, Saini S, Bhatia D, Gupta A, Puraswani M, et al. Serum-soluble urokinase receptor levels do not distinguish focal segmental glomerulosclerosis from other causes of nephrotic syndrome in children. Kidney Int. 2014;85(3):649–58.
7. Meijers B, Maas RJH, Sprangers B, Claes K, Poesen R, Bammens B, et al. The soluble urokinase receptor is not a clinical marker for focal segmental glomerulosclerosis. Kidney Int. 2014;85:636–40.
8. Wada T, Nangaku M, Maruyama S, Imai E, Shoji K, Kato S, et al. A multicenter cross-sectional study of circulating soluble urokinase receptor in Japanese patients with glomerular disease. Kidney Int. 2014;85:641–8.
9. Matsuo S, Imai E, Horio M, Yasuda Y, Tomita K, Nitta K, et al. Revised equations for estimated GFR from serum creatinine in Japan. Am J Kidney Dis. 2009;53:982–92.
10. The Japanese Society of Nephrology. Guidelines for the treatment of nephrotic syndrome. Nihon Jinzo Gakkai Shi. 2011;53:78–122.
11. The Japanese Society of Nephrology. Guidelines for the treatment of rapidly progressive glomerulonephritis. Nihon Jinzo Gakkai Shi. 2011;53:509–55.
12. Maas RJ, Wetzels JF, Deegens JK. Serum-soluble urokinase receptor concentration in primary FSGS. Kidney Int. 2012;81:1043–4.
13. Maas RJ, Deegens JK, Wetzels JF. Serum suPAR in patients with FSGS: trash or treasure? Prediatr Nephrol. 2013;28:1041–8.
14. Zang B, Shi W, Ma J, Sloan A, Faul C, Wei C, et al. The calcineurin-NFAT pathway allows for urokinase receptor-mediated β3 integrin signaling to cause podocyte injury. J. Mol. Med. (Berl.). 2012;90:1407–20.
15. Bock ME, Price H, Gallon L, Langman CB. Serum soluble urokinase-type plasminogen activator receptor levels and idiopathic FSGS in children: A single-center reports. Clin Am Soc Nephrol. 2013;8:1304–11.

16. Backes Y, van der Sluijs KF, Mackie DP, Tacke F, Koch A, Tenhunen JJ, et al. Usefulness of suPAR as a biological marker in patients with systemic inflammation or infection: a systematic review. Intensive Care Med. 2012;38:1418–28.
17. Koch A, Voigt S, Kruschinski C, Sanson E, Duckers H, Horn A, et al. Circulating soluble urokinase plasminogen activator receptor is stably elevated during the first week of treatment in the intensive care unit and predicts mortality in critically ill patients. Crit Care. 2011;15:R63.
18. Yilmaz G, Koksal I, Karahan SC, Mentese A. The diagnostic and prognostic significance of soluble urokinase plasminogen activator receptor in systematic inflammatory response syndrome. Clin Biochem. 2011;44:1227–30.
19. Baraldi A, Zambruno G, Furci L, Ballestri M, Tombesi A, Ottani D, et al. β_1 and β_3 integrin upregulation in rapidly progressive glomerulonephritis. Nephrol Dial Transplant. 1995;10:1155–61.

Correspondence: *Takehiko Wada or Masaomi Nangaku, Division of Nephrology and Endocrinology, University of Tokyo School of Medicine, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan.*
E-mail: twada-tky@umin.ac.jp or mnangaku-tky@umin.ac.jp

Kidney International (2014) **86,** 208–209; doi:10.1038/ki.2014.139

Serum levels of soluble urokinase plasminogen activator receptor in Japanese patients with chronic kidney disease

To the Editor: We read with interest the recent article by Wada *et al.*[1] on the role of soluble urokinase plasminogen activator receptor (suPAR) levels in Japanese patients with focal segmental glomerulosclerosis (FSGS) and chronic kidney disease (CKD). They demonstrated that suPAR levels were significantly affected by renal function and were not effective in discriminating FSGS from other glomerular diseases, indicating that suPAR levels are not a potent diagnostic marker for clinical use in Japanese patients. They did not, however, describe the relationship between serum suPAR levels and the progression of renal injury in Japanese CKD patients. Given that the clinical significance of serum suPAR in CKD is unclear, we studied the relationship between serum suPAR and various clinical parameters in 487 Japanese CKD patients. In addition to measuring the basal levels of serum clinical parameters, including suPAR and creatinine (Cr) levels, estimated glomerular filtration rate (eGFR), and urine protein to Cr ratio, we also evaluated serum and urine samples collected after 1 and 2 years ($n=208$). Similarly, our results show that suPAR levels are inversely correlated with eGFR ($P<0.0001$, $r=-0.275$), and positively associated with CKD stage ($P<0.0001$) and urine protein to Cr ratio ($P<0.0001$, $r=0.157$; Figure 1a–c), indicating that renal function significantly affects suPAR levels. Notably, with respect to the relationship between basal suPAR levels and the progression rate of kidney function (ΔeGFR = [final eGFR − initial eGFR]/initial eGFR), suPAR levels were inversely associated with ΔeGFR in samples from year 1 ($P<0.0001$, $r=-0.148$; Figure 1d) and year 2 ($P=0.0002$, $r=-0.126$; Figure 1e).

Consistent with our results, Meijers *et al.*[2] recently reported that suPAR was not a clinical marker for FSGS patients in Europe. In contrast, Wei *et al.*[3] and Shankland *et al.*[4] indicated the importance of circulating suPAR levels as a cause of FSGS in the United States. Although these discrepancies might be explained by ethnic differences, further global studies are required. In Japanese CKD patients, suPAR might therefore be a predictive marker of renal function, and basal levels of suPAR might help to predict the progression of renal injury.

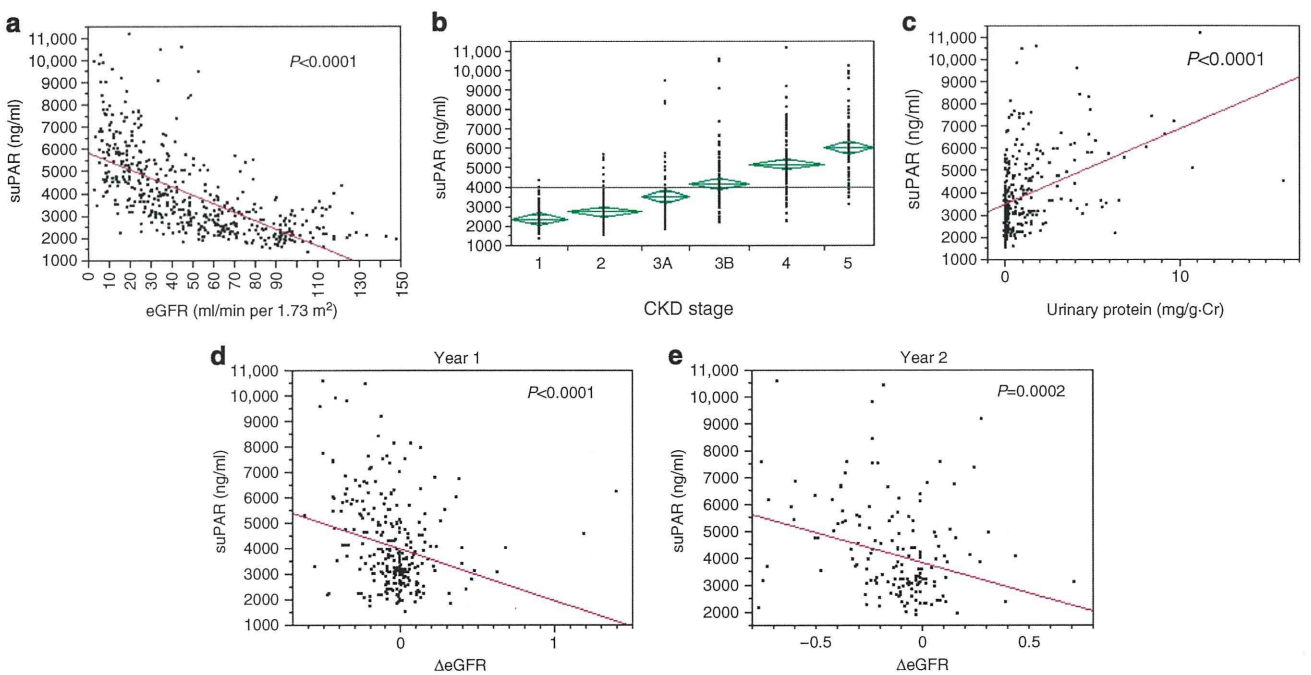

Figure 1 | Relationship between soluble urokinase plasminogen activator receptor (suPAR) levels and estimated glomerular filtration rate (eGFR), chronic kidney disease (CKD) stage, urinary protein, and the rate of change of eGFR for years 1 and 2 in Japanese CKD patients. suPAR levels were inversely correlated with eGFR ($P<0.0001$, $r=-0.275$; **a**), and positively associated with CKD stage ($P<0.0001$; **b**) and urine protein to creatinine (Cr) ratio ($P<0.0001$, $r=0.157$; **c**). The relationship between basal suPAR levels and the progression rates of kidney function (ΔeGFR = [final eGFR − initial eGFR]/initial eGFR): suPAR levels were inversely associated with ΔeGFR in samples from year 1 ($P<0.0001$, $r=-0.148$; **d**) and year 2 ($P=0.0002$, $r=-0.126$; **e**).

ACKNOWLEDGMENTS
This study was supported by grants from the Japan Osteoporosis Foundation, Japanese Kidney Foundation, Kochi Organization for Medical Reformation and Renewal (YT), and a grant from the Ministry of Education, Science, Culture and Sports of Japanese government (YS, SF, and YT).

1. Wada T, Nangaku M, Maruyama S *et al.* A multicenter cross-sectional study of circulating soluble urokinase receptor in Japanese patients with glomerular disease. *Kidney Int* 2014; **85**: 641–648.
2. Meijers B, Maas RJ, Sprangers B *et al.* The soluble urokinase receptor is not a clinical marker for focal segmental glomerulosclerosis. *Kidney Int* 2014; **85**: 636–640.
3. Wei C, El Hindi S, Li J *et al.* Circulating urokinase receptor as a cause of focal segmental glomerulosclerosis. *Nat Med* 2011; **17**: 952–960.
4. Shankland SJ, Pollak MR. A suPAR circulating factor causes kidney disease. *Nat Med* 2011; **17**: 927–927.

Yoshinori Taniguchi[1], Yoshiko Shimamura[1], Taro Horino[1], Shimpei Fujimoto[1] and Yoshio Terada[1]

[1]*Department of Endocrinology, Metabolism and Nephrology, Kochi University School of Medicine, Nankoku, Japan*
Correspondence: *Yoshinori Taniguchi or Yoshio Terada, Department of Endocrinology, Metabolism and Nephrology, Kochi University School of Medicine, Kohasu, Oko-cho, Nankoku, Kochi 783-8505, Japan.*
E-mail: taniguchiy@kochi-u.ac.jp or terada@kochi-u.ac.jp

Kidney International (2014) **86,** 209–210; doi:10.1038/ki.2014.136

The Authors Reply: We thank Taniguchi *et al.*[1] for their interest in our recent paper. In their cohort of 487 Japanese patients with chronic kidney disease (CKD), several interesting and important findings came to light.

Their study included CKD patients regardless of underlying renal diseases, and it should be noted that a significant number of their patients had severe CKD. Moreover, unlike our cross-sectional study,[2] they collected follow-up data from 208 patients. Their first finding was the inverse correlation at baseline between serum soluble urokinase plasminogen activator receptor (suPAR) levels and estimated glomerular filtration rate (eGFR), which supports our data[2] and others.[3,4] Next they showed that serum levels of suPAR were positively associated with urinary protein excretion, whereas we did not find any association between serum suPAR levels and proteinuria in our multi-center national cohort. A potential explanation for this discrepancy is that urinary protein excretion and renal function could be potential confounding factors for each other in the study population including severe CKD patients. Finally, they demonstrated that suPAR levels were inversely associated with the progression rate of kidney function, ΔeGFR, in year 1 as well as in year 2. Although a potential role of suPAR levels as a predictive factor of CKD progression seems fascinating, we cannot exclude the possibility that eGFR at baseline affected their data, given that mild CKD patients should have lower levels of suPAR and that the proportion of stable patients may be larger in a mild CKD population. The roles of suPAR as a pathological factor or as a diagnostic marker in CKD are ambiguous.

1. Taniguchi Y, Shimamura Y, Horino T *et al.* Serum levels of soluble urokinase plasminogen activator receptor in Japanese patients with chronic kidney disease. *Kidney Int* 2014; **86**: 209–210.
2. Wada T, Nangaku M, Maruyama S *et al.* A multicenter cross-sectional study of circulating soluble urokinase receptor in Japanese patients with glomerular disease. *Kidney Int* 2014; **85**: 641–648.
3. Huang J, Liu G, Zhang YM *et al.* Plasma soluble urokinase receptor levels are increased but do not distinguish primary from secondary focal segmental glomerulosclerosis. *Kidney Int* 2013; **84**: 366–372.
4. Meijers B, RJH Maas, Sprangers B *et al.* The soluble urokinase receptor is not a clinical marker for focal segmental glomerulosclerosis. *Kidney Int* 2014; **85**: 636–640.

Takehiko Wada[1], Masaomi Nangaku[1], Shoichi Maruyama[2] and Seiichi Matsuo[2]

[1]*Division of Nephrology and Endocrinology, University of Tokyo School of Medicine, Tokyo, Japan and* [2]*Department of Nephrology, Nagoya University Graduate School of Medicine, Nagoya, Japan*
Correspondence: *Masaomi Nangaku or Takehiko Wada, Division of Nephrology and Endocrinology, University of Tokyo School of Medicine, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan.*
E-mail: mnangaku-tky@umin.ac.jp or twada-tky@umin.ac.jp

Kidney International (2014) **86,** 210; doi:10.1038/ki.2014.138

Rituximab in pure red-cell aplasia secondary to anti-erythropoietin antibodies

To the Editor: MacDougall *et al.*[1] provided a comprehensive review of pure red-cell aplasia (PRCA) induced by antibodies directed against erythropoietin (EPO) and its treatments (steroids, cyclosporin A (CsA), or cyclophosphamide). However, they have overlooked evidence for treatment by rituximab (RTX), a monoclonal antibody directed against $CD20^+$ B cells. In May 2012, we made a diagnosis of antibody-mediated PRCA in a 69-year-old male followed for short bowel syndrome, and requiring total parenteral nutrition. For the last 36 months, he had received epoetin-α to treat anemia related to mild myelodysplastic syndrome (MDS) and moderate renal failure (glomerular filtration rate 47 ml/min per 1.73 m^2). As short bowel syndrome precluded adequate enteric absorption of steroids and CsA and cyclophosphamide was contraindicated because of underlying MDS, intravenous RTX was deemed the most appropriate therapy. RTX was administered intravenously (1 g at days 1 and 15; June 2012). One month after RTX, anti-EPO antibody levels decreased substantially from 69 to 5 IU/ml (<0.1 IU/ml at month 14 despite CD19+ B-cell recovery). From June to August 2012, patient received iterative RBC transfusion (Figure 1), while one transfusion was required thereafter.

Consistent with previous reports, including one of successful re-treatment by EPO,[2,3] the very short delay