Soluble Tyro-3 and Axl may reflect the current activity and renal involvement in patients with microscopic polyangiitis and granulomatosis with polyangiitis

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Abstract

Objective

This study investigated whether soluble Tyro-3 (sTyro-3), sAxl, and sMer could reflect the current activity in patients with microscopic polyangiitis (MPA) and granulomatosis with polyangiitis (GPA).

Methods

This study retrospectively reviewed the medical records of 76 patients with MPA and GPA, and measure the serum concentrations of sTyro-3, sAxl, and sMer using the stored serum at AAV diagnosis. Antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV)-specific indices included Birmingham vasculitis activity index (BVAS), five-factor score, the short-form 36-item health survey, and vasculitis damage index. High AAV activity was defined as the highest tertile of BVAS.

Results

The median age of the 47 MPA and 29 GPA patients was 66.0 years and 43.4% were men. The serum concentrations of sTyro-3 and sAxl were significantly correlated with BVAS and the total score of renal manifestation. The serum concentrations of sTyro-3 and sAxl were independently correlated with BVAS (β=0.343 and β=0.310, respectively). In addition, the serum concentrations of sTyro-3 and sAxl were independently associated with the renal involvement of MPA and GPA (OR 1.003 and OR 1.055, respectively).

Conclusion

This study demonstrated the potential of the serum concentrations of sTyro-3 and sAxl to reflect the current activity and renal involvement in patients with MPA and GPA.

Key words

sTyro-3, sAxl, microscopic polyangiitis, granulomatosis with polyangiitis, activity

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Introduction

TAM receptors (Tyro-3, Axl, and Mer) are members of receptor protein tyrosine kinases. TAM receptors have been demonstrated to participate in vascular homeostasis including proliferation, apoptosis, efferocytosis, leukocyte migration, and platelet aggregation (1). Growth arrest-specific gene 6 (Gas-6), one of the ligands of TAM receptors, has a similar structure to protein S, which has a gamma-carboxyl glutamic acid domain; however, it has not been identified to be involved in the coagulation cascade (2). The Gas-6-induced activation of Axl is known to be stronger than those of Tyro-3 and Mer; nevertheless, those of Tyro-3 and Mer can be potentiated by apoptotic cells and enveloped viruses (3). The extracellular domains of Tyro-3, Axl, and Mer can be effectively cleaved by disintegrin and metalloprotease (ADAM) 10 and 17 spontaneously or through their increased expression (4, 5). Among the cleaved forms of TAM receptors, soluble Tyro-3 (sTyro-3) and soluble Axl (sAxl) are known to play a role as antagonists leading to switching off the Gas-6-induced activation of TAM receptors-mediated signalling more preferentially than soluble Mer (sMer) (3). The immunological mechanisms via the TAM receptor-mediated signalling have been identified to play anti-inflammatory roles by regulating macrophage functions as follows: i) enhancing efferocytosis; ii) diminishing type I interferon-mediated responses, iii) suppressing the autophagy-induced nucleotide-binding oligomerisation domain, leucine-rich repeat, pyrin domain-containing (NLRP)3 inflammasome activation, and iv) inhibiting M1 macrophage polarisation (6). Therefore, when the TAM receptor-mediated signalling is normally activated or circulating concentrations of the soluble forms of TAM receptors (sTAM) are decreased, self-immune tolerance may be maintained by these anti-inflammatory actions. Whereas, when TAM receptor-mediated signalling is insufficiently activated or circulating concentrations of sTAM increase, self-immune tolerance may be disrupted, leading to the development of autoimmunity (7). According to the previous studies, it was reported that the plasma concentrations of sAxl and sMer were associated with the severity of lupus nephritis in patients with systemic lupus erythematosus, and those of sMer were elevated in patients with anti-SSA/Ro-positive Sjogren syndrome (8, 9).

Antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) is a small-vessel vasculitis, which is characterised by necrotising vasculitis in capillaries, arterioles, venules, and occasionally medium-sized arteries (10). AAV is divided into three subtypes according to clinical, laboratory, radiological, and histopathological features: microscopic polyangiitis (MPA), granulomatosis with polyangiitis (GPA), and eosinophilic GPA (EGPA) (11-15). Given the mechanisms of action of TAM receptor-mediated signalling, and the pathogenesis of AAV, it can be reasonably assumed that sTyro-3, sAxl, and sMer may be positively associated with the current activity of AAV. Under these concepts, there was a study demonstrating that the serum concentrations of sAxl were consistently higher in patients with active EGPA than in those with inactive EGPA (16). However, till now, no studies have investigated the potential of sTyro-3, sAxl, and sMer to reflect the current activity of MPA and GPA. Hence, in this study, we investigated whether sTyro-3, sAxl, and sMer could reflect the current activity in patients with MPA and GPA.

Patients and methods

Patients

We selected 76 patients with MPA and GPA from the Severance Hospital ANCA-associated VasculitidEs (SHAVE) cohort (a prospective observational cohort of patients with AAV). All the patients were classified as having MPA or GPA according to the 2007 European Medicines Agency algorithms for AAV and polyarteritis nodosa, and the 2012 revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides (10, 11). The additional inclusion criteria of this study were i) patients who were reclassified as having MPA and GPA according to

Table I. Characteristics of patients with MPA and GPA (n=76).

Variables	Values	
Demographic data		
Age (years)	66.0	(18.8)
Male sex (n, (%))	33	(43.4)
AAV subtypes (n, (%))		
MPA	47	(61.8)
GPA	29	(38.2)
ANCA positivity (n, (%))		
MPO-ANCA (or P-ANCA) positive	46	(60.5)
PR3-ANCA (or C-ANCA) positive	11	(14.5)
Systemic items of BVAS (n, (%))		
General manifestation	31	(40.8)
Cutaneous manifestation	13	(17.1)
Mucous and ocular manifestation	3	(3.9)
Otorhinolaryngologic manifestation	23	(30.3)
Pulmonary manifestation	51	(67.1)
Cardiovascular manifestation	6	(7.9)
Gastrointestinal manifestation	1	(1.3)
Renal manifestation	50	(65.8)
Nervous systemic manifestation	19	(25.0)
Total scores of systemic items of BVAS		
General manifestation	0	(2.0)
Cutaneous manifestation	0	(0)
Mucous and ocular manifestation	0	(0)
Otorhinolaryngologic manifestation	0	(1.0)
Pulmonary manifestation	3.0	(4.0)
Cardiovascular manifestation	0	(0)
Gastrointestinal manifestation	0	(0)
Renal manifestation	6.0	(12.0)
Nervous systemic manifestation	0	(0.8)
AAV-specific indices		
BVAS	12.0	(12.0)
SF-36 PCS	45.9	(35.6)
SF-36 MCS	50.3	(30.8)
VDI	3.0	(2.0)
Routinely tested laboratory results		
White blood cell count (/mm^3)	8,640.0	(6,190.0)
Neutrophil count (/mm^3)	6,210.0	(6,410.0)
Lymphocyte count (/mm^3)	1,400.0	(970.0)
Haemoglobin (g/dL)	10.6	(4.3)
Platelet count (x1,000/mm^3)	271.0	(180.0)
Blood urea nitrogen (mg/dL)	21.5	(20.3)
Serum creatinine (mg/dL)	0.9	(1.5)
Total protein (g/mL)	6.6	(0.9)
Serum albumin (g/dL)	3.7	(1.2)
Alkaline phosphatase (IU/L)	76.0	(37.0)
Aspartate transaminase (IU/L)	19.0	(10.0)
Alanine transaminase (IU/L)	17.0	(14.0)
Acute phase reactants		
ESR (mm/hr)	45.0	(78.0)
CRP (mg/L)	7.0	(59.2)
Serum concentrations of sTAM (pg/mL)		
sTyro-3	2,496.0	(2,728.0)
sAxl	106.8	(40.4)
sMer	1,914.0	(1,082.0)

Values are expressed as a median (interquartile range) or n (%).
MPA: microscopic polyangiitis; GPA: granulomatosis with polyangiitis; ANCA: antineutrophil cytoplasmic antibody; AAV: ANCA-associated vasculitis; MPO: myeloperoxidase; P: perinuclear; PR3: proteinase 3; C: cytoplasmic; BVAS: Birmingham vasculitis activity score; SF36: 36-item short form survey; PCS: physical component summary; MCS: mental component summary; VDI: vasculitis damage index; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; TAM: Tyro-3, Axl, and Mer; s: soluble.

the 2022 American College of Rheumatology (ACR)/European Alliance of Associations for Rheumatology (EULAR) classification criteria for MPA and GPA (13, 14); ii) patients who had sufficient medical records for collecting clinical, laboratory, radiological, and histopathological data; iii) patients in whom the Birmingham vasculitis activity index (BVAS, version 3) (17), the Korean version of the Short-Form 36-Item Health Survey (SF-36) physical component summary (PCS) and mental component summary (MCS) (18), and vasculitis damage index (VDI) (19) were assessed at the time of AAV diagnosis; iv) patients who did not have serious medical conditions other than AAV such as malignancies and severe infectious diseases; v) patients who had never received glucocorticoids more than 20mg/day or immunosuppressive drugs for AAV treatment within the first month before AAV diagnosis to avoid the factors for confusing AAV diagnosis or activity assessment. This study was approved by the Institutional Review Board (IRB) of Severance Hospital (4-2016-0901) and, when required, written informed consent was obtained when required from patients at the time of blood sampling. IRB waived the need for written informed consent when it had been previously obtained at entry into the SHAVE cohort.

Clinical data and high AAV activity

The clinical data at the time of AAV were collected in this study to minimise the confounding factors (Table I). The titres of myeloperoxidase (MPO)-ANCA and proteinase 3 (PR3)-ANCA were measured by an immunoassay, the novel anchor-coated highly sensitive (HS) Phadia ELiA (Thermo Fisher Scientific/Phadia, Freiburg, Germany) and human native antigens, on the Phadia250 analyser. According to the 2022 ACR/EULAR criteria for MPA and GPA, both perinuclear (P)-ANCA and cytoplasmic (C)-ANCA detected by an indirect immunofluorescence assay and MPO-ANCA or PR3-ANCA measured by an immune assay were considered ANCA positivity (13, 14). Systemic manifestations were based on BVAS items, and AAV-specific indices

Table II. Correlation of serum concentrations of sTAM with AAV-specific indices and total score of systemic items of BVAS in patients with MPA and GPA.

		sTyro-3	sAxl	sMer	BVAS	SF-36 PCS	SF-36 MCS	VDI	Ge	Cu	M	E	P	Ca	Ga	R	N
sTyro-3	r	1	0.492	0.126	0.303	0.160	0.102	0.226	0.148	-0.115	-0.088	-0.019	0.170	-0.032	-0.069	0.505	-0.166
	p^*		*<0.001*	*0.278*	*0.008*	*0.170*	*0.382*	*0.049*	*0.203*	*0.324*	*0.451*	*0.870*	*0.143*	*0.782*	*0.557*	*<0.001*	*0.151*
sAxl	r	0.492	1	-0.028	0.433	-0.030	-0.004	0.161	0.110	0.056	-0.170	-0.182	0.091	0.072	0.108	0.569	0.066
	p	*<0.001*		*0.809*	*<0.001*	*0.798*	*0.973*	*0.165*	*0.342*	*0.631*	*0.143*	*0.115*	*0.433*	*0.537*	*0.354*	*<0.001*	*0571*
sMer	r	0.126	-0.028	1	0.031	-0.033	-0.166	-0.050	0.150	-0.072	-0.018	0.066	0.129	-0.021	-0.050	-0.028	-0.009
	p	*0.278*	*0.809*		*0.790*	*0.779*	*0.323*	*0.668*	*0.365*	*0.538*	*0.879*	*0.569*	*0.266*	*0.859*	*0.666*	*0.808*	*0.940*

p^*: *p*-value.
s: soluble; TAM: Tyr-3, Axl, and Mer; AAV: ANCA-associated vasculitis; ANCA: antineutrophil cytoplasmic antibody; BVAS: Birmingham vasculitis activity score; MPA: microscopic polyangiitis; GPA: granulomatosis with polyangiitis; SF36: 36-item short form survey; PCS: physical component summary; MCS: mental component summary; VDI: vasculitis damage index; Ge: general manifestation; Cu: cutaneous manifestation; M: mucous and ocular manifestation; E: ear nose throat (Otorhinolaryngologic) manifestation; P: pulmonary manifestation; Ca: cardiovascular manifestation; Ga: gastrointestinal manifestation; R: renal manifestation; N: nervous systemic manifestation; r: correlation coefficient.

included BVAS, SF-36 PCS, MCS, and VDI (17-19).

Blood samples and measurement of sTyro-3, sAxl, and sMer

Whole blood was obtained from patients who provided consent on the same day that the assessments of AAV-specific indices were completed. Serum was immediately isolated from whole blood and stored at -80°C. The serum concentrations of sTyro3, sAxl, and sMer were measured from stored sera by ELISA kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The sensitivity of sTyro3, sAxl, and sMer is 4.6, 7.457, and 6.96 pg/mL, respectively.

Statistical analyses

All statistical analyses were performed using IBM SPSS Statistics for Windows, version 26 (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as medians with interquartile ranges, whereas categorical variables were expressed as numbers (percentages). The correlation coefficient (r) between the two variables was obtained using either Pearson's correlation analysis or a univariable linear regression analysis. The standardised correlation coefficient (β) was obtained by a multivariable linear regression analysis using variables with statistical significance in the univariable analysis. The odds ratio (OR) was obtained using the multivariable logistic regression analysis including variables with $p<0.005$ in the univariable logistic regression analysis. The relative risk (RR) of the cut-off of sAxl for high AAV activity was analysed using contingency tables and the chi-square test. The statistical significance was set at $p<0.05$.

Results

Characteristics of 76 patients with MPA and GPA

The median age of the 47 MPA and 29 GPA patients was 66.0 years and 43.4% were men. MPO-ANCA (or P-ANCA) and PR3-ANCA (or C-ANCA) were detected in 46 and 11 patients, respectively. The most common systemic item of BVAS was pulmonary manifestation (67.1%), followed by renal manifestation (65.8%). The median BVAS, SF-36 PCS, MCS, and VDI scores were 12.0, 45.9, 50.3, and 3.0, respectively. The median serum concentrations of sTyro-3, sAxl, and sMer were 2,496.0 pg/mL, 106.8 pg/mL, and 1,914.0 pg/mL respectively (Table I).

Correlation analyses

First, among sTyro-3, sAxl, and sMer, the serum concentrations of sTyro-3 were significantly correlated with those of sAxl (r=0.492, $p<0.001$). Regarding AAV-specific indices, the serum concentrations of sTyro-3 (r=0.303, $p=0.008$) and sAxl (r=0.433, $p<0.001$) were significantly correlated with BVAS, whereas only the serum concentrations of sTyro-3 (r=0.226, $p=0.049$) were significantly correlated with VDI, despite a low statistical power. Regarding the total scores of systemic items of BVAS, the serum concentrations of sTyro-3 (r=0.505, $p<0.001$) and sAxl (r=0.569, $p<0.001$) were significantly correlated with the total score of renal manifestation. However, no significant correlation was not observed among the serum concentrations of sMer, AAV-specific indices, and the total score of systemic items of BVAS (Table II).

Linear regression analysis for BVAS

In the univariable linear analysis, white blood cell count ($p=0.001$), alkaline phosphatase ($p=0.018$), erythrocyte sedimentation rate (ESR) ($p<0.001$), and C-reactive protein (CRP) ($p=0.004$) were positively correlated with BVAS, whereas haemoglobin ($p<0.001$), total protein ($p=0.025$), and serum albumin ($p<0.001$) were negatively correlated with BVAS. The serum concentrations of sTyro-3 ($p=0.008$) and sAxl ($p<0.001$) were significantly correlated with BVAS. In the multivariable linear analysis with the serum concentrations of sTyro-3, white blood cell count (β=0.283, $p=0.004$), haemoglobin (β=-0.444, $p=0.001$), and the serum concentrations of sTyro-3 (β=0.343, $p<0.001$) were independently correlated with BVAS. Alternatively, in the multivariable linear analysis with the serum concentrations of sAxl, white blood cell count (β=0.249, $p=0.013$), haemoglobin (β=-0.447, $p=0.002$), and the serum concentrations of sAxl (β=0.310, $p=0.002$) were also independently correlated with BVAS (Table III).

Table III. Linear regression of sTyro-3 or sAxl and continuous laboratory variables for the current BVAS in patients with MPA and GPA.

Variables	Univariable			Multivariable (sTyro-3)			Multivariable (sAxl)		
	Beta	95% CI	*p*-value	Beta	95% CI	P value	Beta	95% CI	*p*-value
Age (years)	0.142	-0.048, 0.203	0.220						
White blood cell count (/mm^3)	0.376	0.266, 0.998	0.001	0.283	0.174, 0.873	0.004	0.249	0.100, 0.821	0.013
Neutrophil count (/mm^3)	0.126	-0.122, 0.407	0.285						
Lymphocyte count (/mm^3)	-0.174	-0.797, 0.114	0.139						
Haemoglobin (g/dL)	-0.628	-2.654, -1.458	<0.001	-0.444	-2.332, -0.613	0.001	-0.447	-2.378, -0.592	0.002
Platelet count (x1,000/mm^3)	0.144	-0.005, 0.019	0.222						
Blood urea nitrogen (mg/dL)	0.228	-0.001, 0186	0.053						
Serum creatinine (mg/dL)	-0.170	-0.150, 0.130	0.886						
Total protein (g/mL)	-0.264	-4.706, -0.320	0.025	-0.112	-3.664, 1.507	0.407	-0.139	-4.040, 1.353	0.322
Serum albumin (g/dL)	-0.600	-8.714, -4.534	<0.001	-0.072	-4.368, 2.805	0.664	0.011	-3.662, 3.905	0.949
Alkaline phosphatase (IU/L)	0.280	0.007 0.074	0.018	-0.022	-0.032, 0.026	0.834	-0.075	-0.041, 0.020	0.498
Aspartate transaminase (IU/L)	-0.015	-0.174, 0.153	0.899						
Alanine transaminase (IU/L)	0.030	-0.075, 0.096	0.804						
ESR (mm/hr)	0.427	-0.040, 0.124	<0.001	0.254	-0.006, 0.102	0.080	0.245	-0.010, 0.103	0.103
CRP (mg/L)	0.341	0.017, 0.087	0.004	-0.089	-0.054, 0.027	0.501	-0.093	-0.056, 0.028	0.495
Serum concentrations of sTAM									
sTyro-3 (pg/mL)	0.303	0.001, 0.005	0.008	0.343	0.002, 0.005	<0.001			
sAxl (pg/mL)	0.433	0.042, 0.121	<0.001				0.310	0.023, 0.098	0.002
sMer (pg/mL)	0.031	0.000, 0.000	0.790						

s: soluble; BVAS: Birmingham vasculitis activity score; MPA: microscopic polyangiitis; GPA: granulomatosis with polyangiitis; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; TAM: Tyro-3, Axl, and Mer.

Table IV. Logistic regression of sTyro-3 or sAxl and continuous laboratory variables for renal involvement in patients with MPA and GPA.

Variables	Univariable			Multivariable (Tyro-3)			Multivariable (Axl)		
	OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value
Age (years)	1.040	1.004, 1.076	0.027	1.016	0.947, 1.091	0.657	0.987	0.907, 1.074	0.756
Male gender (N, (%))	2.250	0.827, 6.119	0.112						
MPO-ANCA (or P-ANCA) positive	3.182	1.188, 8.524	0.021	1.327	0.205, 8.595	0.767	1.341	0.151, 11.937	0.792
PR3-ANCA (or C-ANCA) positive	0.370	0.101, 1.357	0.134						
White blood cell count (/mm^3)	1.151	1.014, 1.307	0.030	1.313	0.992, 1.739	0.057	1.142	0.874, 1.491	0.331
Neutrophil count (/mm^3)	1.015	0.942, 1.094	0.698						
Lymphocyte count (/mm^3)	0.567	0.269, 1.195	0.136						
Haemoglobin (g/dL)	0.622	0.486, 0.796	<0.001	0.512	0.241, 1.084	0.080	0.466	0.186, 1.163	0.102
Platelet count (x1,000/mm^3)	0.999	0.996, 1.002	0.445						
Blood urea nitrogen (mg/dL)	1.177	1.077, 1.287	<0.001	1.182	1.022, 1.368	0.025	1.195	1.024, 1.394	0.024
Serum creatinine (mg/dL)	0.993	0.959, 1.027	0.669						
Total protein (g/mL)	0.553	0.293, 1.041	0.067						
Serum albumin (g/dL)	0.228	0.096, 0.542	0.001	0.595	0.069, 5.157	0.637	1.469	0.099, 21.778	0.780
Alkaline phosphatase (IU/L)	1.006	0.993, 1.019	0.371						
Aspartate transaminase (IU/L)	0.978	0.939, 1.020	0.297						
Alanine transaminase (IU/L)	0.993	0.972, 1.015	0.526						
ESR (mm/hr)	1.013	1.000, 1.026	0.055						
CRP (mg/L)	1.005	0.995, 1.016	0.334						
Serum concentrations of sTAM									
sTyro-3 (pg/mL)	1.002	1.001, 1.003	0.001	1.003	1.001, 1.005	0.014			
sAxl (pg/mL)	1.063	1.033, 1.094	<0.001				1.055	1.016, 1.095	0.006
sMer (pg/mL)	1.000	1.000, 1.000	0.660						

s: soluble; BVAS: Birmingham vasculitis activity score; MPA: microscopic polyangiitis; GPA: granulomatosis with polyangiitis; OR: odds ratio; MPO: myeloperoxidase; ANCA: antineutrophil cytoplasmic antibody; P: perinuclear; PR3: proteinase 3; C: cytoplasmic; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; TAM: Tyro-3, Axl, and Mer.

Logistic regression analysis for renal involvement

In the univariable logistic analysis, age (p=0.027), MPO-ANCA (or P-ANCA) positivity (p=0.021), white blood cell count (p=0.030), haemoglobin (p<0.001), blood urea nitrogen (p<0.001), serum albumin (p=0.001), and the serum concentrations of sTyro-3 (p=0.001) and sAxl (p<0.001) were significantly associated with the renal involvement of MPA and GPA. In the multivariable logistic analysis with the serum concentrations of sTyro-3, blood urea nitrogen (OR 1.182, 95% CI 1.022, 1.368) and the serum concentrations of sTyro-3 (OR 1.003, 95% CI 1.001, 1.005) were independently associated with the renal involvement of MPA and GPA. In the multivariable logistic analysis with the serum con-

centrations of sAxl, blood urea nitrogen (OR 1.195, 95% CI 1.024, 1.394) and the serum concentrations of sAxl (OR 1.055, 95% CI 1.016, 1.095) were independently associated with the renal involvement of MPA and GPA (Table IV).

Discussion

The motivation for conducting this study was the following theoretical background: the impaired phagocytosis function of dendritic cells and macrophages is one of the main points in the AAV mechanism. TAM receptors, however, play a crucial role in phagocytosis by binding to their ligand such as Gas-6. High concentrations of sTyro-3 and sAxl might cause a malfunction in phagocytosis by hindering the activation of TAM receptors (3, 6, 12). The results of this study enabled inferring the immuno-clinical role of sTyro-3 and sAxl in the pathophysiology of MPA and GPA as follows: first, the exacerbation of MPA and GPA might accelerate the expression of ADAM 10 and 17 (cleaving enzymes), resulting in an increase in the serum concentrations of sTyro-3 and sAxl (20); second, the augmented serum concentrations of sTyro-3 and sAxl might inhibit the binding of Gas-6 to the membranous forms of Tyro-3 and Axl and offset their anti-inflammatory effect, leading to maximising the proinflammatory proportion and forming a vicious cycle that worsens the activity of MPA and GPA (3); and third, unlike the patterns of the contribution of all three types of sTAM to the pathophysiology of lupus nephritis (21-24), only sTyro-3 and sAxl might be associated with both the current activity and the renal involvement of MPA and GPA.

Among the three types of sTAM, sTyro-3 and sAxl were significantly associated with the current BVAS (Table III). Given that the Tyro-3 and Axl-mediated signalling is closely related to self-immune tolerance (7), it may be assumed that developing an agonist to accelerate the Tyro-3 and Axl-mediated signalling can alleviate the severity of AAV by augmenting its anti-inflammatory property. Alternatively, it can be speculated that developing a monoclonal antibody to hinder binding between sTAM receptors and Gas-6 may be useful in controlling AAV (3, 6). However, promoting Tyro-3 and Axl-mediated signalling may provoke unwanted cancers. Until recently, anti-cancer drug development targeting these TAM receptors was actively pursued (25). In theory, Tyro-3 and Axl-inhibitory agents may reduce their signalling, leading to diminished T cell immune tolerance and enhanced T cell surveillance, thereby preventing cancer from occurring (7). Therefore, sTyro-3 and sAxl may have clinical significance only as biomarkers for reflecting the current activity or the renal involvement of MPA and GPA but not as a therapeutic candidate.

In general, serum creatinine is expected to show a strong association with the renal involvement of AAV. However, in the univariable logistic regression analysis in Table IV, serum creatinine was not significantly associated with the renal involvement of MPA and GPA, unlike blood urea nitrogen. These results might be due to the differently weighted points assigned to detailed items of BVAS: four, four, and six points are assigned to hypertension, proteinuria > +1, and haematuria ≥10 red blood cells/high power field, respectively, in cases of newly developed renal manifestations. Whereas, different scores are assigned to serum creatinine only once according to its level ranges: four, six, or eight points for 1.41-2.82 mg/dL, 2.83-5.64 mg/dL, and ≥5.66 mg/dL, respectively. Because data at the time of diagnosis were used in this study, the proportion of those with chronic renal disease stage 4 or 5 was not likely to be high, and thus, there were only 5 patients who received 6 points assigned to an item of rise in serum creatinine >30% or fall in creatinine clearance >25% (17). Therefore, since the ratio of serum creatinine in the item of renal manifestation of BVAS was about 1/4 (hypertension, proteinuria, haematuria, and serum creatinine), serum creatinine might have shown no significant association with the renal involvement of MPA and GPA.

In addition, serum creatinine is a metabolite of creatine phosphate of muscle and thus is closely related to total muscle mass. Because muscle mass is often reduced in elderly patients, serum creatinine may not accurately reflect renal function. In other words, serum creatinine could be measured lower despite poor renal function (26). The median age of the patients included in this study was 66.0 years and the median serum creatinine was 0.9 mg/dL. Therefore, the possibility that serum creatinine underestimated renal function could not be ruled out, and for this reason, it is presumed that blood urea nitrogen showed a more significant association with the renal involvement of MPA and GPA than serum creatinine.

We wondered about the differences in the serum concentrations of sTAM receptors according to the AAV subtype and ANCA type. First, we compared the serum concentrations of sTyro, sAxl, and sMer between GPA and MPA patients. We found that MPA patients exhibited a significantly higher median serum concentration of sAxl than GPA patients (122.1 pg/mL *vs*. 83.5 pg/mL, $p<0.001$), whereas, there were no significant differences in the serum concentrations of sTyro and sMer between the two groups (Supplementary Fig. S1). Next, we compared the serum concentrations of sTAM receptors according to the presence of MPO-ANCA (or P-ANCA) or PR3-ANCA (or C-ANCA). We found that patients with MPO-ANCA (or P-ANCA) exhibited significantly higher median serum concentrations of sTyro-3 and sAxl than those without (2,274.0 pg/mL *vs*. 1,606.0 pg/mL, $p=0.009$ for sTyro-3, and 118.2 pg/mL *vs*. 94.1 pg/mL, $p=0.033$ for sAxl). There were no significant differences in the serum concentrations of sTAM receptors between patients with PR3-ANCA (or C-ANCA) and those without (Supplementary Fig. S2).

Using the variables with statistical significance in the multivariable logistic analysis of either sTyro-3 or sAxl for the renal involvement of MPA and GPA (Table IV), we developed two equations for estimating the renal involvement. We assigned a weight of a number close to an integer to each variable according to the slopes for the independent variable with a *p* value

<0.05 in the multivariable analysis as described in our previous study (27). The new equations are as follows: i) equation 1 (sAxl) = 0.178 × (blood urea nitrogen) + 0.053 × (sAxl), and ii) equation 2 (sTyro-3) = 0.167 × (blood urea nitrogen) + 0.003 × (sTyro-3). Using the ROC curve, the AUC of equation 1, equation 2, sAxl, and sTyro-3 were compared. Equation 1 exhibited the highest AUC (area 0.941, 95% CI 0.889, 0.992), followed by sAxl (area 0.899, 95% CI 0.820, 0.978) and equation 2 (area 0.865, 95% CI 0.784, 0.946) (Supplementary Fig. S3). From these results, two conclusions can be drawn. First, the renal involvement of MPA and GPA can be estimated using the two newly developed equations without urinalysis to detect haematuria and proteinuria. Their efficiencies were superior to or comparable to that of sAxl alone. Second, sAxl appeared to be more sensitive to estimating the renal involvement of MPA and GPA than sTyro-3.

This study has several limitations. Most importantly, the number of patients was not large enough to not only perform subgroup analyses, but also generalise the results of this study. The retrospective study design was also another major limitation with concerns about the quality of clinical data and stored serum samples. In addition, cystatin C could have minimised the possibility that serum creatinine might underestimate poor renal function in elderly patients (28); however, it could not be used in this study due to the limited number of patients with the results of cystatin C at the time of diagnosis. In particular, we did not measure the serum concentrations of sTAM receptors in EGPA patients in this study due to a limitation of the number of samples. If it is possible, it may provide valuable information on the comparison of their clinical implication among three subtypes of AAV. However, as a pilot study, this study has the clinical implications in that it is the first to demonstrate that the serum concentrations of both sTyro-3 and sAxl were significantly and independently correlated with the current activity of MPA and GPA and associated with the renal involvement, and that that the optimal cut-off of the serum concentrations of sAxl could predict their high AAV activity. It is believed that a prospective future study with more patients will overcome these limitations and provide dynamic and more reliable clinical implications for the serum concentrations of sTyro-3 and sAxl in patients with MPA and GPA.

In conclusion, this study demonstrated the potential of the serum concentrations of sTyro-3 and sAxl to reflect the current activity and the renal involvement in patients with MPA and GPA.

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