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Original Article

Clinical and Applied Thrombosis/Hemostasis
Volume 26: 1-9
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DOI: 10.1177/1076029620938874
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Plasma Level of von Willebrand Factor Propeptide at Diagnosis: A Marker of Subsequent Renal Dysfunction in Autoimmune Rheumatic Diseases

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Abstract

Introduction: Patients with systemic autoimmune rheumatic diseases (SARDs) such as rheumatoid arthritis, systemic lupus erythematosus (SLE), Sjögren syndrome, and systemic sclerosis, which are chronic inflammatory diseases, are prone to develop renal dysfunction, which is related to vascular endothelial cell damage. **Material and Methods:** We evaluated plasma levels of von Willebrand factor (VWF), VWF propeptide (VWF-pp), disintegrin-like and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS13), and VWF multimer pattern in patients with SARDs at diagnosis and investigated whether they may serve as markers to identify patients destined to develop renal dysfunction within 1 year. Renal dysfunction was defined as subsequent reduced estimated glomerular filtration rate (eGFR) by >25% or the new appearance of abnormal urine findings such as proteinuria (protein > 30 mg/dL) or hematuria (red blood cells >20/HPF in urine sediments). Overall, 63 patients with SARDs were studied. **Results and Conclusions:** We observed a significant increase of VWF-pp and a significant decrease of ADAMTS13 in patients with SARDs compared with normal healthy controls. The highest level of VWF-pp was observed in patients with SLE among the groups. The levels of VWF and multimer pattern of VWF were not different compared with normal healthy controls. Von Willebrand factor propeptide predicted a subsequent decrease in eGFR at a cutoff point of 210% (sensitivity, 78.6%; specificity, 73.5%) and new urinary abnormal findings at a cutoff point of 232% (sensitivity, 77.8%; specificity, 77.8%) Using these cutoff points, multivariable analysis revealed that VWF-pp was a significant risk factor for renal dysfunction at an odds ratio of 8.78 and 22.8, respectively, and may lead to a new therapeutic approach to prevent vasculitis and renal dysfunction.

Keywords

von Willebrand factor propeptide, ADAMTS13, renal dysfunction, SLE, mutimeric analysis, rheumatic disease

Date received: 16 March 2020; revised: 26 May 2020; accepted: 10 June 2020.

Introduction

Von Willebrand factor (VWF) is a plasma multimeric glycoprotein that plays a pivotal role in primary hemostasis by mediating adhesion and aggregation of platelets on collagen exposed at the site of vascular injury.[1] Recent studies have indicated that VWF not only regulates hemostasis but also inflammation, the immune system, and angiogenesis.[2,3]

Von Willebrand factor is synthesized as pre–pro-VWF consisting of a signal peptide, propeptide, and a mature VWF monomer in endothelium and undergoes extensive intracellular modification. Pro-VWF monomers form C-terminal dimers,

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and then these pro-VWF dimers are assembled into large multimers while releasing VWF propeptide (VWF-pp). Von Willebrand factor is stored in Weibel-Palade bodies in the endothelium as ultra-large multimeric forms of VWF and VWF-pp in an equimolar basis.[4] Both multimeric VWF and VWF-pp can be released into the blood stream when the endothelium is stimulated. Thus, plasma levels of VWF and VWF-pp can be markers of vascular endothelial activation and are also involved in dysfunction and injury.[5] After secretion from the endothelium, ultra-large multimer VWF (UL-VWF) undergoes a reduction in size through prompt proteolysis by VWF-cleaving protease, a disintegrin-like and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS13).[6] Thus, VWF multimers exist in various sizes in plasma, including high-molecular-weight (HMW), intermediate- molecular-weight, and low-molecular-weight (LMW) VWF. Because the activity of VWF is directly proportional to its length, UL-VWF is the most potent in tethering platelets. Therefore, a deficiency of ADAMTS13 function—such as thrombotic thrombocytopenic purpura (TTP)—perpetuates UL-VWF in circulation, which leads to the formation of intravascular platelet thrombi resulting in thrombocytopenia and organ dysfunction.[7-9]

Although HMW-VWF has more potent activity in hemostasis than LMW-VWF, HMW-VWF is more susceptible to proteolysis by ADAMTS13 than LMW-VWF. Thus, ADAMTS13 is important for maintaining the normal function and size distribution of VWF multimers. Yet, just as ADAMTS13 can control VWF function by reducing its size,[9] VWF can control ADAMTS13. In fact, a study has shown that an infusion of desmopressin decreased the plasma level of ADAMTS13.[10] The researchers suggested that the desmopressin-induced release of higher multimers of VWF led to a consumption of ADAMTS13. Considering that HMW multimeric VWF released from damaged endothelium will reduce levels of ADAMTS13,[10] investigation into the correlation of HMW-VWF and ADAMTS13 levels may help to estimate the extent of vascular endothelial injury in diseases such as vasculitis.

Systemic autoimmune rheumatic diseases (SARDs) such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), Sjögren syndrome (SjS), systemic sclerosis (SSc), myositis, and antineutrophil cytoplasmic antibody-related vasculitis, among others, are chronic inflammatory diseases that are caused by an abnormal immune response to certain environmental triggers as a defensive response in individuals with an appropriate genetic background. Such chronic inflammation can result in organ dysfunction including nephropathy, interstitial pneumonia, hepatitis, and so on, which can be risk factors for morbidity and mortality in SARDs. Therefore, preventing, recognizing, and managing the progression of organ dysfunction are critical in SARDs. For example, in SLE, managing renal involvement (lupus nephritis, LN) is especially important, because LN can progress to end-stage renal disease (ESRD), which can be the main risk factor for SLE-related mortality and morbidity.[11] Nevertheless, despite potent anti-immunosuppressive and anti-inflammatory therapies, ESRD cannot always be avoided.[11] Therefore, identifying patients with SLE who are destined to develop LN is imperative in the early stages of lupus. If those patients can be identified earlier, they can be followed up closely and given potent anti-inflammatory, immunomodulator, immunosuppressant, or other medicines. Currently, it is not possible to determine a priori who with SLE will develop LN using biomarkers related to neutrophil activation, inflammation, complement activation, and the immune complex (IC).[11] So, we speculated that as levels of VWF-ADAMTS13 axis-related molecules reflect initial activation of the coagulation system, they may be useful to identify those patients.

The current study was conducted to investigate whether plasma levels of vascular endothelial injury markers including VWF, VWF-pp, HMW-VWF, and ADAMTS13 can be differentiated among patients with SARDs and be used to identify which patients are destined to develop subsequent renal dysfunction.

Materials and Methods

Patient Selection

The included patients were diagnosed according to the criteria of the American College of Rheumatology as having SARDs, including RA, SLE, SjS, and SSc, at Nara Medical University from January 2007 to March 2015. Patients whose follow-up period was less than 1 year or who already had renal dysfunction (estimated glomerular filtration rate [eGFR] <45 mL/min) were excluded. The study design was approved by the ethical committee of Nara Medical University (approval number: 1413).

Clinical Data

Age, sex, eGFR, and urine findings (proteinuria and hematuria) were evaluated and examined over a year period. Erythrocyte sedimentation rate (ESR); levels of complements including C3, C4, and 50% hemolytic complement activity (CH50); IC; and anti-double stranded DNA antibody (anti-dsDNA Ab) on diagnosis were retrieved from the medical records for patients with SLE.

Measurements of VWF, VWF-pp, ADAMTS13, and HMW-VWF (High Multimer Index) Levels

Blood samples were obtained from study patients on the day of their diagnosis with SARDs. Blood samples were centrifuged at 1500 *g* for 10 minutes and aliquots of plasma were stored at −80 °C until assayed. Measurement of VWF antigen (VWF:Ag; VWF) was performed according to the manufacturer instructions (Dako). The plasma level of VWF-pp antigen was also measured using a commercial kit (Sanquin) according to the manufacturer instruction. We created a standard curve using the normal plasma pooled by healthy volunteers (controls). The ADAMTS13 antigen was also assayed using a

Table 1. Clinical Characteristics of Patients With SARDs.[a]

Characteristic	RA (n = 25)	SLE (n = 10)	SjS (n = 17)	SSc (n = 11)	*P* value
Age, year	59 (53-68)[b]	48 (27-54)[b]	62 (49-70)[b]	59 (54-68)[b]	.056
Sex, male, n (%)	6 (24.0)	1 (10.0)	1 (5.9)	1 (9.1)	.454
eGFR at diagnosis, mL/min/1.73 m^2	80 (70-89)[b]	92 (78-126)[b]	76 (67-93)[b]	79 (70-88)[b]	.206
Proteinuria[c] at diagnosis, n (%)	3 (12.0)	2 (20.0)	0 (0)	0 (0)	.153
Hematuria[d] at diagnosis, n (%)	2 (8.0)	2 (20.0)	2 (11.7)	1 (9.1)	.775
Survival at 1 year, n (%)	25 (100)	10 (100)	17 (100)	11 (100)	NS

Abbreviations: eGFR, estimated glomerular filtration rate; IQR, interquartile range; NS, not significant; RA, rheumatoid arthritis; SARDs, systemic autoimmune rheumatic diseases; SjS, Sjögren syndrome; SLE, systemic lupus erythematosus; SSc, systemic sclerosis.
[a]N = 63.
[b]Median (IQR).
[c]In-urine protein > 30 mg/dL.
[d]In-urine red blood cell > 20/HPF.

commercial kit (Kainos Laboratories Inc). The VWF multimer analysis was performed as described elsewhere.[12] High multimers (HMW-VWF) were defined as chains of more than 10 oligomers, and the proportion of high multimers was calculated as the high multimer ratio from the area under the curve (AUC) drawn by densitometer (the ratio of the high multimers area to the total area of the multimers). Then, the high multimer ratio of the patient was compared with that of a normal control as high multimer index (HMI; high multimer ratio of the patient/high multimer ratio of normal control × 100). These values were compared with normal healthy volunteers (controls) and among the study groups with SARDs.

Subsequent Renal Dysfunction

Renal condition can sometimes improve quickly with various treatments, so decrements in renal function that the treating physicians considered to be due to SARDs were regarded as renal dysfunction. Subsequent renal dysfunction was defined as reduced eGFR by >25% or the new appearance of abnormal urine findings such as proteinuria (protein >30 mg/dL in urine) or hematuria (red blood cells > 20/HPF in urine sediments) within 1 year. We investigated whether the levels of VWF, VWF-pp, ADAMTS13, and HMI at diagnosis before treatment were associated with developing subsequent renal dysfunction within 1 year.

Statistical Analysis

The Mann-Whitney *U* test was used for comparison between 2 groups. The Kruskal-Wallis test was used for comparison between 3 or more groups. Receiver operating characteristic (ROC) analysis was used for determining the most suitable cutoff point of VWF, VWF-pp, ADAMTS13, and HMI levels for distinguishing between patients with or without subsequent renal dysfunction. Odds ratios (ORs) for subsequent renal dysfunction were estimated using binomial logistic regression model. Age, sex, VWF-pp, VWF, ADAMTS13, and HMI were included in a multivariable logistic regression analysis as the independent variables. Von Willebrand factor, VWF-pp, ADAMTS13, and HMI were divided into the high-level group and low-level group using the cutoff point calculated by ROC analysis, and they were put into a multivariable logistic regression analysis as category variables.

All *P* values were 2-sided, and *P* value <.05 was considered as a statistically significant difference.

Statistical analyses were mainly performed using Easy R version 1.3.6.[13]

Results

Baseline Characteristics

A total of 63 patients (RA, n = 25; SLE, n = 10; SjS, n = 17; SSc, n = 11) were included. No significant differences were noted in age, sex, eGFR, or prevalence of proteinuria or hematuria at diagnosis among the groups. Also, no differences were noted in survival rate at 1 year among the 4 groups (Table 1).

Levels of VWF, VWF-pp, ADAMTS13, and HMI

Plasma levels of VWF-pp at diagnosis for each disease were significantly increased compared with controls. The ADAMTS13 levels were significantly decreased in all groups compared with controls. The VWF and HMI levels in each group were not significantly different compared with controls. The VWF-pp level in SLE at diagnosis was significantly higher than that in other diagnoses ($P = .0001$, median of VWF-pp was 300.9 [interquartile range, IQR, 264.4-407.7] in SLE, 145.6 [IQR 116.2-180.7] in RA, 163.5 [IQR 143.8-190.6] in SjS, and 192.4 [IQR 138.6-233.1] in SSc). However, no differences were noted in VWF, ADAMTS13, and HMI levels among the groups. There were extremely high levels of VWF-pp observed in 2 patients with RA who also had rheumatoid vasculitis or mixed connective tissue disease (Figure 1).

Analysis of ROC for Subsequent Renal Dysfunction

The ROC analysis revealed that the AUC of VWF-pp was the largest for subsequent reduced eGFR, and the cutoff point was 210% (sensitivity 78.6%, specificity 73.5%; Figure 2). Also, the cutoff points of VWF, ADAMTS13, and HMI for subsequent reduced eGFR were 138%, 66%, and 124%, respectively.

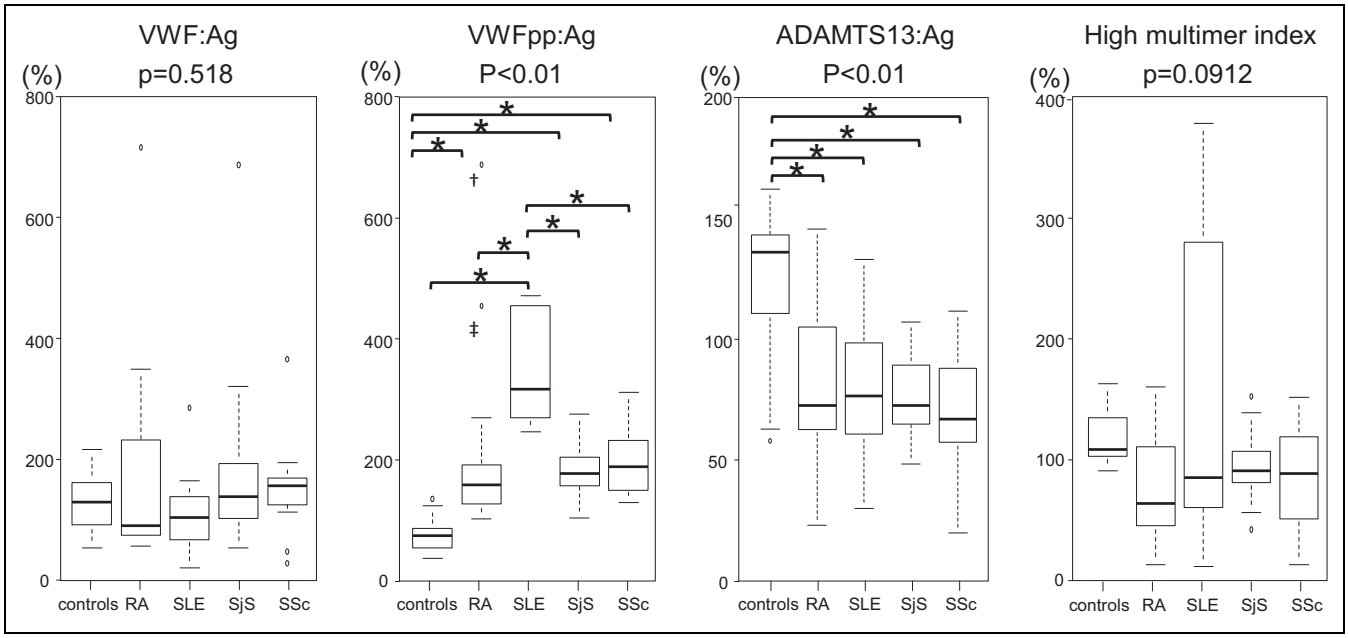

Figure 1. VWF, VWF-pp, ADAMTS13, and high multimer index in patients with SARDs at diagnosis (N = 63). *$P < .01$. A P value on each figure was calculated through comparison among the groups using Kruskal-Wallis test. †Rheumatoid vasculitis with RA. ‡Mixed connective tissue disease with RA. [a]High multimer ratio = area of high multimers[b]/area of all multimers. [b] High multimers: ≥ 11 bands on analysis of VWF multimers. ADAMTS13:Ag indicates a disintegrin-like and metalloproteinase with thrombospondin type 1 motifs 13 antigen; high multimer index (%), high multimer ratio[a] of patients/high multimer ratio of normal controls × 100; RA, rheumatoid arthritis; SARDs, systemic autoimmune rheumatic diseases; SjS, Sjögren syndrome; SLE, systemic lupus erythematosus; SSc, systemic sclerosis; VWF:Ag, von Willebrand factor antigen; VWF-pp:Ag, von Willebrand factor propeptide antigen.

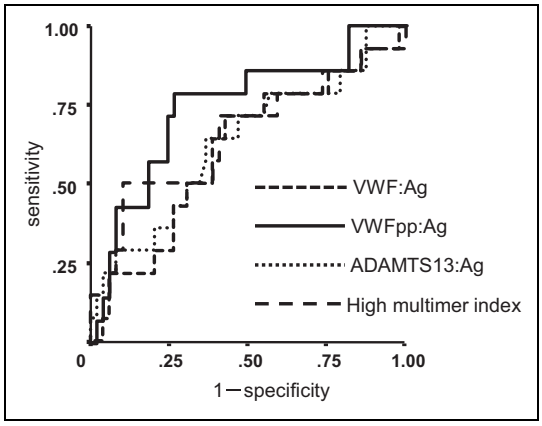

Figure 2. Receiver operating characteristic analysis of VWF, VWF-pp, ADAMTS13, and high multimer index in patients with SARDs for subsequent reduced eGFR[a] (N = 63). VWF:Ag: AUC, 0.603 (95% CI: 0.429-0.778); cutoff point: 138% (sensitivity 71.4%, specificity 57.1%). VWF-pp:Ag: AUC, 0.743 (95% CI: 0.587-0.900); cutoff point: 210% (sensitivity 78.6%, specificity 73.5%). ADAMTS13:Ag: AUC, 0.627 (95% CI: 0.445-0.804); cutoff point: 66% (sensitivity 64.3%, specificity 63.3%). High multimer index: AUC, 0.655 (95% CI, 0.467-0.842); cutoff point: 124% (sensitivity 50.0%, specificity 89.8%). [a]Subsequent reduced eGFR: reduced eGFR by > 25% versus at diagnosis. ADAMTS13:Ag indicates a disintegrin-like and metalloproteinase with thrombospondin type 1 motifs 13 antigen; AUC, area under the curve; eGFR, estimated glomerular filtration rate; ROC, receiver operating characteristic; SARDs, systemic autoimmune rheumatic diseases; VWF:Ag, von Willebrand factor antigen; VWF-pp:Ag, von Willebrand factor propeptide antigen.

Similarly, the AUC of VWF-pp was the largest for subsequent new appearance of abnormal urine findings, and the cutoff point was 232% (sensitivity 77.8%, specificity 77.8%). Also, the cutoff points of VWF, ADAMTS13, and HMI were 126%, 71%, and 70% in the ROC analysis, respectively (Figure 3).

Odds Ratio for Subsequent Reduced eGFR

In the univariable analysis estimated using binomial logistic regression model for subsequent eGFR reduction, VWF-pp (OR: 10.2 [95% CI: 2.44-42.2], $P = .00144$) and HMI (OR: 8.80 [95% CI: 2.18-35.6], $P = .00229$) were significant variable factors. In the multivariable analysis performed using a binomial logistic regression model for subsequent reduced eGFR, VWF-pp was found to be a significant variable factor in age, sex, VWF, ADAMTS13, and HMI adjusted models (OR: 8.78; 95% CI: 1.54-50.0; $P = .0144$; Table 2). Similarly, HMI was an independent risk factor for subsequent reduced eGFR (OR: 5.67; 95% CI: 1.16-27.6; $P = .0318$; Table 2).

Odds Ratio for Subsequent Appearance of New Abnormal Urine Findings

In the univariable analysis using binomial logistic regression model for subsequent new abnormal urine findings, VWF-pp was a significant variable factor (OR: 12.2 [95% CI, 2.24-66.9], $P = .00381$). Also, VWF-pp was a significant variable factor

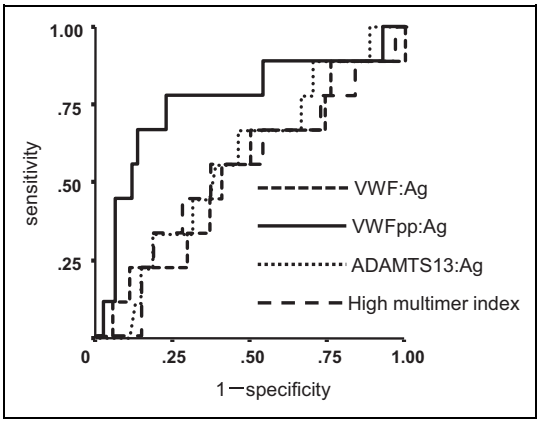

Figure 3. Receiver operating characteristic analysis of VWF, VWF-pp, ADAMTS13, and high multimer index in patients with SARDs for subsequent new abnormal urine[a] findings (N = 63). VWF:Ag: AUC, 0.529 (95% CI: 0.310-0.747); cutoff point: 126% (sensitivity 66.7%, specificity 50.0%). VWF-pp:Ag: AUC, 0.765 (95% CI: 0.559-0.972); cutoff point: 232% (sensitivity 77.8%, specificity 77.8%). ADAMTS13:Ag: AUC, 0.570 (95% CI: 0.373-0.767); cutoff point: 71% (sensitivity 66.7%, specificity 53.7%). High multimer index: AUC, 0.535 (95% CI: 0.317-0.753); cutoff point: 70% (sensitivity 55.6%, specificity 63.0%). [a]Subsequent new abnormal urine findings: new appearance of proteinuria and hematuria. ADAMTS13:Ag indicates a disintegrin-like and metalloproteinase with thrombospondin type 1 motifs 13 antigen; AUC, area under the curve; ROC indicates receiver operating characteristic; SARDs, systemic autoimmune rheumatic diseases; VWF:Ag, von Willebrand factor antigen; VWF-pp:Ag, von Willebrand factor propeptide antigen.

Table 2. Multivariable ORs for Subsequent Reduced eGFR.[a]

	Subsequent reduced eGFR[b]		
Independent variables	OR	95% CI	*P* value
Model 1[c]			
Age, year	1.01	0.956-1.06	.774
Sex, male	0.275	0.019-3.98	.344
ADAMTS13 < 66%	1.10	0.211-5.75	.908
VWF > 138%	1.21	0.253-5.82	.808
VWF-pp > 210%	8.78	1.54-50.0	.0144
High multimer index[d] > 124%	5.67	1.16-27.6	.0318

Abbreviations: ADAMTS13, a disintegrin-like and metalloproteinase with thrombospondin type 1 motifs 13 antigen; OR, odds ratio; VWF, von Willebrand factor antigen; VWF-pp, von Willebrand factor propeptide antigen.
[a]N = 63.
[b]Subsequent reduced eGFR: reduced eGFR by over 25% within 1 year compared to that on diagnosis.
[c]Model 1 simultaneously included age, sex, VWF, VWF-pp, ADAMTS13, and high multimer index as independent variables.
[d]High multimer index (%) = high multimer ratio[e] of patients/high multimer ratio of normal controls × 100.
[e]High multimer ratio = area of high multimers[f]/area of all multimers.
[f]High multimers: ≥11 bands on analysis of VWF multimers.

for the subsequent appearance of new abnormal urine findings in the multivariable analysis adjusted by age and sex, VWF, ADAMTS13, and HMI (OR: 22.8; 95% CI: 2.36-221.0; $P = .00692$; Table 3).

Table 3. Multivariable ORs for Subsequent New Abnormal Urine Findings.[a]

	Subsequent new abnormal urine findings[b]		
Independent variables	OR	95% CI	*P* value
Model 1[c]			
Age, year	0.972	0.916-1.03	.344
Sex, male	3.98	0.431-36.8	.223
ADAMTS13 < 71%	0.137	0.0122-1.55	.108
VWF > 126%	6.27	0.748-52.6	.0906
VWF-pp > 232%	22.8	2.36-221.0	.00692
High multimer index[d] > 70%	0.227	0.0237-2.17	.198

Abbreviations: ADAMTS13, a disintegrin-like and metalloproteinase with thrombospondin type 1 motifs 13 antigen; OR, odds ratio; VWF, von Willebrand factor antigen; VWF-pp, von Willebrand factor propeptide antigen.
[a]N = 63.
[b]Subsequent abnormal urine findings: new appearance of proteinuria and hematuria within 1 year (proteinuria: in-urine protein > 30 mg/dL, hematuria: in-urine red blood cell > 20/HPF).
[c]Model 1 simultaneously included age, sex, VWF, VWF-pp, ADAMTS13, and high multimer index as independent variables.
[d]High multimer index (%) = high multimer ratio[e] of patients/high multimer ratio of normal controls × 100.
[e]High multimer ratio = area of high multimers[f]/area of all multimers.
[f]High multimers: ≥ 11 bands on analysis of VWF multimers.

Table 4. Relationship Between Subsequent Renal Dysfunction and VWF-pp, ESR, Complement, IC, and anti-dsDNA Ab in Patients With SLE.[a,b]

	Subsequent renal dysfunction[c]		
	Positive (n = 7)	Negative (n = 3)	*P* value
VWF-pp, %	249.0 (211.5-304.5)	157.3 (134.3-217.9)	.00683
ESR, mm	45.0 (27.5-56.5)	38.0 (31.0-42.0)	.648
C3, mg/mL	69.2 (58.3-108.1)	87.2 (82.8-89.2)	.833
C4, mg/mL	10.7 (6.25-16.6)	11.4 (10.3-11.7)	.667
CH50, CH50/mL	30.0 (21.5-42.5)	31.0 (30.5-31.5)	.909
IC, μg/mL	1.50 (1.50 -1.50)	1.50 (1.50-2.05)	.416
Anti-dsDNA Ab, IU/mL	10.0 (10.0-30.5)	10.0 (6.0-10.0)	.521

Abbreviations: anti-dsDNA Ab, anti-double stranded DNA antibody; C3, complement 3; C4, complement 4; CH50, 50% hemolytic complement activity; eGFR, estimated glomerular filtration rate; ESR, erythrocyte sedimentation rate; IC, immune complex; IQR, interquartile range; SLE, systemic lupus erythematosus; VWF-pp, von Willebrand factor propeptide antigen.
[a]n = 10.
[b]Values presented as median (IQR).
[c]Subsequent renal dysfunction: reduced eGFR by >25% within 1 year compared with at diagnosis.

Relationship Between Subsequent Renal Dysfunction and VWF-pp, ESR, Complements, IC, and anti-dsDNA Ab in Patients With SLE

In the analysis of patients with SLE (n = 10), VWF-pp was significantly higher in patients who developed subsequent renal dysfunction than patients who did not develop renal dysfunction ($P = .00683$; Table 4). No differences were noted in ESR,

complement levels, IC, and anti-dsDNA Ab titer among the groups.

Discussion

We investigated VWF, VWF-pp, ADAMTS13, and HMI levels in patients with SARDs (RA, SLE, SjS, and SSc). The main results of this study were as follows. First, compared with controls, all patients with an SARD had significantly increased levels of VWF-pp and significantly decreased levels of ADAMTS13 at diagnosis (Figure 1). These biomarkers did not show any difference among the groups, except VWF-pp, which was significantly higher in the SLE group compared with the other groups. Second, increased levels of VWF-pp were significantly associated with subsequent renal dysfunction, suggesting that VWF-pp level may identify patients destined to develop subsequent renal dysfunction.

Because VWF plays an important role in hemostasis by tethering platelets to the subendothelial matrix, an increased level of VWF has been regarded as a thrombosis risk and has been reported to be associated with risks for coronary heart disease, ischemic stroke, venous thrombosis, and cerebral sinus thrombosis.[14] Also, VWF level has been considered to be a biomarker for the severity of vascular disorders including arteritis, hypertension, and connective tissue disease.[14,15] Nevertheless, we did not find significant elevation of VWF level at diagnosis in all SARDs group compared with controls. In contrast, Habe et al[16] reported that VWF level was increased in patients with connective tissue disease including RA, SLE, SSc, SjS, and so on. Considering that endothelium stimulation may be mediated by autoantibodies or IC in patients with SARDs, such patients may have increased production and release of VWF to circulation, resulting in increased plasma levels of VWF. The reason why VWF was not elevated in our cases may be that the degree of vascular endothelial stimulation is less than in their cases because we measured VWF at the time of diagnosis. Furthermore, VWF level can be influenced by consumption by hemostatic reaction, by blood type, and by many established cardiovascular risk factors such as age, cholesterol levels, and hypertension,[14] which may complicate use of VWF level as a biomarker in clinical practice.[17]

Because VWF-pp is cosecreted with VWF from endothelium on an equimolar basis, has a shorter half-life (2-3 hours) than VWF (8-12 hours),[18] and is less involved in maintaining physiological homeostasis than VWF, VWF-pp was proposed to as a better predictor of acute vascular endothelial injury.[5,19]

Thus, we focused on VWF-pp level as a better biomarker for vascular endothelial injury than VWF. Several reports have demonstrated that VWF-pp level is increased in sepsis,[20] diabetes,[21] malaria,[22] connective tissue diseases,[16] among other conditions. Indeed, our results indicated that VWF-pp level was significantly increased in patients with SARDs compared with controls, suggesting that even patients with RA or SjS who had few vascular complications may also have endothelial cell damage at diagnosis. These findings are supported by at least one similar study, which found that vascular endothelial cell damage was observed not only in patients with SLE but also in patients with RA and was present from the early stage of the disease.[23] We observed the highest level of VWF-pp in patients with SLE compared with the other SARDs (RA, SjS, and SSc), suggesting the existence of heightened vascular endothelial disturbance, perhaps due to vasculitis, which may lead to organ dysfunction like LN. Interestingly, 2 patients had extremely high levels of VWF-pp in the RA group, and they were complicated cases with rheumatoid vasculitis and mixed connective tissue disease, respectively. They may be harboring severe vascular endothelial cell damage that likely caused vasculitis and increased VWF-pp levels.

The ADAMTS13 level was significantly decreased in patients with SARDs compared with normal healthy volunteers (Figure 1). No differences in ADAMTS13 level were noted among individuals with SARDs. The cause of decreased level of ADAMTS13 has not been elucidated. Mannucci et al[24] reported that the level of ADAMTS13 was decreased in 123 patients with SLE or SSc, and the inhibitors to ADAMTS13 were not found. It suggests that the presence of inhibitors to ADAMTS13 may not be the cause of the low levels of ADAMTS13 in SARDs. Furthermore, decreased production of ADAMTS13 is also unlikely to be the mechanism responsible for low ADAMTS13 because none of the patients in our study had severe liver failure. Considering the high levels of VWF-pp in patients with SARDs, excessive VWF release from endothelium in such patients may induce consumption of ADAMTS13, as mentioned earlier, resulting in decreased level of ADAMTS13.

High levels of VWF release and low levels of ADAMTS13 may be involved in the pathophysiology of many diseases. Indeed, the imbalance between VWF and ADAMTS13 has been demonstrated to be associated with the pathophysiology of stroke severity,[25] progression of liver cirrhosis,[26] dementia risk,[27] severity of pediatric trauma,[28] sepsis,[20] cardiovascular mortality,[29] and risk of myocardial infarction.[30] These associations may suggest that the extent of vascular endothelial cell damage might correlate with the severity of various diseases. Although patients with SARDs did not have higher levels of VWF than controls in our study, the imbalance between high level of VWF release (estimated from high level of VWF-pp) and low level of ADAMTS13 may be a possible contributor to the progression of organ dysfunction in SARDs, because the imbalance can be prothrombotic, to some extent reminiscent of what happens during TTP.

The severe and persistent vascular endothelial cell damage may result in persistent secretion of VWF, leading to consumably depletion of ADAMTS13 activity and then the appearance of UL-VWF. Indeed, decreased level of ADAMTS13 in patients with sepsis or after nonelective cardiac surgery was also reported to be associated with the appearance of UL-VWF in plasma.[31] Thus, the presence of UL-VWF can be a marker for severe forms of SARDs. Based on these observations, we did a multimeric analysis of VWF to investigate the distribution of multimers of VWF in patients with SARDs. This study is the first report of multimeric analysis in SARDs. Bockmeyer

et al[32] reported that HMW-VWF was increased in a porcine septic model 12 hours after sepsis induction, with decreased levels of ADAMTS13 and platelet thrombosis in the kidney. This finding may indicate that increased secretion of UL-VWF from damaged endothelium by sepsis induction will increase HMW-VWF in the early phases of vascular endothelial cell damage and enhanced proteolysis of VWF, resulting in consumption and decreased levels of ADAMTS13. We speculated that HMI (relative ratio of HMW-VWF) would be increased in patients with SARDs at diagnosis and UL-VWF would be found in the severest form of SARDs. However, we did not find significant increase of HMI and UL-VWF in patients with SARDs compared with controls. The reason for the insignificant increase in HMI and lack of UL-VWF may be because the patients with SARDs were investigated early (at diagnosis), when they may have not had enough serious injury of their endothelium to reduce function of ADAMTS13 resulting in increased HMI and the appearance of UL-VWF. Nevertheless, multivariable analysis revealed that HMI was an independent risk factor for subsequent reduced eGFR, increased HMI can be an indicator for endothelium injury which may be related to renal dysfunction in patients with SARDs.

Next, we investigated the relationship of the levels of VWF-ADAMTS13 axis-related molecules with subsequent renal dysfunction, to date, no biomarkers have been found which are suitable to effectively identify patients with SARDs who are destined to develop renal failure. For example, ESR as an indirect indicator of inflammation, and low levels of complement as indicators of an abnormal immune system in SLE, are usually used to estimate activity of LN, but it is unlikely that these biomarkers will be useful in identifying patients destined to develop LN. Therefore, we focused on the VWF-ADAMTS13 molecules related to the coagulation response—one of the defensive responses other than inflammatory and immune responses—because the coagulation response can also be activated in SARDs and may correlate with the pathophysiological changes of renal dysfunction. Thus, we examined the relationship of VWF, VWF-pp, ADAMTS13, and HMI levels with renal dysfunction. We determined the optimal cutoff point for each biomarker in the ROC analysis for subsequent renal dysfunction. The ROC analysis demonstrated that the AUC of VWF-pp was the highest among the biomarkers, and VWF-pp level over 210% or 232% could predict subsequent reduced GFR or appearance of new abnormal urine findings, respectively, with a sensitivity of 78.6% or 77.8% and a specificity of 73.5% or 77.8% (Figures 2 and 3). Furthermore, VWF-pp was a significant independent risk factor for subsequent reduced eGFR and abnormal urine findings with multivariable analysis in age, sex, ADAMTS13, VWF, and HMI adjusted model, with ORs of 8.78 and 22.8, respectively. These findings suggest that VWF-pp on the day of diagnosis can be a good biomarker to identify patients destined to develop renal dysfunction within 1 year. Von Willebrand factor propeptide may be the best biomarker for vascular endothelial cell damage and the best predictor for subsequent renal dysfunction in SARDs.

To investigate why VWF-pp can predict renal dysfunction, we focused on patients with SLE. We evaluated whether the conventional laboratory markers used to represent SLE inflammatory or immunological activity can differentiate between patients with or without subsequent renal dysfunction. No differences were noted in ESR, C3, C4, CH50, IC, and anti-dsDNA Ab between the patients with or without subsequent renal dysfunction (Table 4), while VWF-pp level did differentiate those patients. This finding means that vascular endothelial cell damage expressed by increased VWF-pp level on the day of diagnosis of SLE may not be caused by inflammatory or immunological changes and may be most associated with the cause of subsequent renal dysfunction.

It is intriguing to think what factors lead to vascular endothelial alterations represented by increased VWF-pp, because suppressing those factors could possibly be a reasonable treatment to prevent subsequent renal dysfunction. Numerous reports have demonstrated that vascular endothelial activation, dysfunction, or injury are associated with disease activity in SLE. These vascular endothelial alterations have been observed in SLE using E-selectin,[33] thrombomoullin,[34] endothelin-1,[35] flow-mediated dilatation,[36] circulating vascular endothelial cells,[37] vascular endothelial microparticles,[38] and so on. Nevertheless, the vascular endothelial alterations expressed by these biomarkers do not always correlate with each other and the level of disease activity of SLE.[37,39] It is possible that VWF-pp level represents the extent of vascular endothelial apoptosis, because vascular endothelial apoptosis also can cause vascular endothelial dysfunction.[39] If so, VWF-pp may be a marker for developing high incidence of atherosclerotic vascular events in patients with SLE.[39]

If we could determine a priori who with SARDs will develop renal dysfunction using VWF-pp level in addition to the conventional biomarkers such as ESR or complement level, such patients could be followed closely and treated better or differently according to the VWF-pp level to prevent renal dysfunction.

Limitations

Some limitations in this study are worth noting. We selected subtle changes in renal function including reduced GFR by more than 25% and appearance of new abnormal urine findings. But these trivial nephropathies may not always be suitable as a predictor or as the first signs of permanent renal dysfunction leading to ESRD. Furthermore, this study was retrospective and limited to only a small number of patients; larger and controlled prospective studies are necessary to validate these findings. In addition, a pathological diagnosis of vasculitis was not determined in all cases.

Conclusion

We found a significantly increased plasma VWF-pp level and a significantly decreased ADAMTS13 level in patients diagnosed with SARDs. The plasma level of VWF-pp in patients

with SARDs at diagnosis may be a more sensitive biomarker for subsequent renal dysfunction within 1 year than VWF, ADAMTS13, and HMI levels and may lead to a new therapeutic approach to prevent vasculitis and renal dysfunction.

Acknowledgments

The authors wish to acknowledge Dr Bolstad Francesco, Professor of the Department of Clinical English, Nara Medical University, for help to edit the manuscript.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported in part by grants from the MEXT KAKENHI (#18K07420 to KN).

Ethical Approval

Ethical approval to report this case series was obtained from ethical committee of Nara Medical University (Approval Number 1413). Written informed consent was obtained from the patients for their anonymized information to be published in this article.

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