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Full Length Article

Complement activation associated with ADAMTS13 deficiency may contribute to the characteristic glomerular manifestations in Upshaw-Schulman syndrome

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ABSTRACT

Introduction: Upshaw-Schulman syndrome (USS) is a congenital form of thrombotic thrombocytopenic purpura (TTP) associated with loss-of-function mutations in the *ADAMTS13* gene, possibly leading to aberrant complement activation and vascular injury. However, USS is extremely rare, and there have been no systematic studies correlating histopathological severity with local *ADAMTS13* expression and complement activation.

Materials and methods: Here, we compared histopathological features, ADAMTS13 immunoreactivity, and immunoreactivity of complement proteins C4d and C5b-9 among renal biopsy tissues from five USS cases, ten acquired TTP cases, and eleven controls.

Results: Pathological analysis revealed chronic glomerular sclerotic changes in the majority of USS cases (4 of 5), with minor glomerular pathology in the remaining case. In two of these four severe cases, more than half of the glomerular segmental sclerosis area was localized in the perihilar region. The average number of ADAMTS13-positive cells per glomerulus was significantly lower in USS cases than controls ($p < 0.05$). Conversely, C4d staining was significantly more prevalent in the glomerular capillary walls of USS cases than controls ($p < 0.05$), while C5b-9 staining did not differ significantly among groups.

Conclusions: These findings suggest that the severity of glomerular injury in USS is associated with deficient ADAMTS13 expression and local complement activation, particularly in vascular regions with higher endothelial shear stress. We suggest that C4d immunostaining provides evidence for complement-mediated glomerular damage in USS.

1. Introduction

Thrombotic thrombocytopenic purpura (TTP) is a rare life-threatening blood disorder caused by severely reduced activity of the metalloprotease ADAMTS13 (a disintegrin-like and metalloproteinase with thrombospondin type 1 motifs 13) [1,2]. There are two TTP subtypes, acquired and hereditary. Acquired TTP is more common and is caused by autoantibodies that block ADAMTS13 activity [3]. Hereditary TTP, also known as Upshaw-Schulman syndrome (USS), results from inherited mutations in *ADAMTS13* that cause severe deficiencies in enzymatic activity (< 10% of wild type) [1,2,4,5]. The incidence of TTP is only 4–10 cases/million people/year, and < 5% of TTP cases are

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classified as USS [6], limiting large-scale studies.

Hepatic stellate cells are a primary site of ADAMTS13 synthesis [7,8]. Expression of ADAMTS13 has been detected at the mRNA and protein levels in cultured renal podocytes, glomerular endothelial cells, and tubular epithelial cells; likewise, ADAMTS13 bioactivity has been demonstrated in these cell types [9–11]. Upshaw-Schulman syndrome is characterized clinically by recurrent episodes of thrombocytopenia and microangiopathic hemolytic anemia responsive to infusions of prophylactic fresh frozen plasma (FFP). Renal dysfunction is a major complication of both USS and acquired TTP, and is manifested histopathologically as thrombotic microangiopathy (TMA) characterized by thrombosis, endothelial cell swelling in small vessels, and glomerular mesangiolysis [12].

Manea et al. reported that signs of acute thrombotic microangiopathy, such as glomerular endothelial swelling and vascular thrombus, are characteristic pathological manifestations of USS [9]. More recently, a causal relationship between TMA and complement activation was proposed in atypical hemolytic uremic syndrome and TTP, including USS [13,14]. However, no study has examined the associations of these pathological features with local ADAMTS13 expression and complement activation. To this end, we conducted simultaneous histopathological examination and immunohistochemical staining for ADAMTS13 and complement proteins in renal biopsy tissues from USS patients and matched controls as well as acquired TTP patients.

2. Materials and methods

2.1. USS patient group

The present study enrolled five cases of USS with available renal biopsy specimens. These biopsy samples were originally obtained from five different hospitals in Japan, and cases 1, 2, 3, and 4 have been previously reported [5,15–18]. In all cases, the diagnosis of USS was based on severely impaired ADAMTS13 activity (< 10% of control) in the absence of ADAMTS13 inhibitors, and mutations in the *ADAMTS13* gene. Patient demographics, laboratory data, and clinical courses are summarized in Table 1 and Fig. 1. Average patient age was 21.2 years (range, 9–40 years) and serum creatinine at the time of renal biopsy was 2.62 mg/dl (range, 0.6–6.16 mg/dl). Serum complement levels in cases 2–5 were within normal limits, while no laboratory data were available for case 1. Proteinuria levels ranged from 0.1 to 0.15 g/dl in all cases with available laboratory data (cases 3–5).

2.2. Control group and acquired TTP cases

The control group consisted of 11 biopsy specimens without severe renal impairment. Six biopsy samples were obtained from patients with mild hematuria or minimal change disease, and histology showed only minor glomerular abnormalities (MGA). Five biopsy samples were obtained from patients with focal segmental glomerulosclerosis with proteinuria. The other one case was obtained from an autopsy case without renal disorders. Eight cases of acquired TTP (three biopsy and five autopsy samples) were also examined. Four cases had proteinuria and hematuria, one case had neither proteinuria nor hematuria, and renal data of three cases were unknown. In control and acquired TTP cases, average ages at the time of renal biopsy were 57.8 years (range, 23–83 years) and 38.9 years (range, 22–66 years), and average serum creatinine levels were 1.15 mg/dl (range, 0.49–2.43 mg/dl) and 3.32 mg/dl (range, 0.78–7.59 mg/dl), respectively.

2.3. Pathological examination

Formalin-fixed, paraffin-embedded tissue sections of renal biopsies were stained with hematoxylin and eosin (HE), periodic acid-Schiff (PAS), periodic acid-methenamine-silver (PAM), and elastica Masson trichrome (EMT). Histological findings of glomerular injury changes and vascular lesions suggestive of TMA were examined. The grade of interstitial fibrosis and tubular atrophy (IF/TA) was evaluated according to the Banff scheme [19]. Arteriosclerosis was evaluated qualitatively according to a three-grade scale (mild, moderate, and severe). The results of electron microscopic examinations and previous immunofluorescence studies (IgA, IgG, IgM, and C3 staining) were retrieved from pathological data records of the original hospitals.

2.4. Immunohistochemistry

Sections were deparaffinized and subjected to antigen retrieval. After blocking endogenous peroxidases, the sections were incubated with primary antibodies overnight at 4 °C, followed by incubation with horseradish peroxidase (HRP)-conjugated secondary antibodies. The primary antibodies used in the present study (and suppliers) were as follows: anti-ADAMTS13 (PA5-14339; Thermo Fisher, Rockford, USA; 1:200), anti-C4d (BI-RC4d; Biomedica Gruppe, Vienna, Austria; 1:50), and anti-C5b-9 (M0777; Dako, Carpinteria, CA, USA; 1:100). Renal arteriolar smooth muscle cells from normal autopsy cases served as positive controls for ADAMTS13 immunostaining [20]. Tissues from cases of antibody-mediated allograft rejection were used as positive controls for Cd4 staining. For C5b-9 immunostaining, renal biopsies of patients with membranous nephropathy served as positive controls. The primary antibody incubation step was omitted for all negative controls.

2.5. Quantitative evaluation of immunohistological results

The number of ADAMTS13-positive cells per glomerulus was counted for all visible glomeruli in each biopsy section. In autopsy samples, 20 glomeruli were examined. Average number of ADAMTS13-positive cells per glomerulus was calculated for quantitative comparisons. Glomerular immunopositivity for C4d or C5b-9 was defined as circumferential tuft staining in at least one capillary. The average numbers of C4d- and C5b-9-positive glomerular capillaries were compared among USS, control, and TTP groups. Immunopositivity for C4d and C5b-9 in arterioles was defined as staining along the luminal side of the vessel. Positivity for C4d in peritubular capillaries was defined according to the Banff 2007 criteria [19].

Table 1
Clinical characteristics of USS at the time of renal biopsy.

Case no.	Patient	Age (years)	Sex	Serum creatinine (mg/dl)	Serum C3 (mg/dl)	Serum C4 (mg/dl)	Proteinuria (g/dl)	ADAMTS13 gene mutations	
								Father's origin	Mother's origin
1	C3	11	M	Unknown	Unknown	Unknown	Unknown	c.414 + 1G > A	c.414 + 1G > A
2	Q1	30	M	6.16	97	39.4	Unknown	p.G227R	p.C908Y
3	U3	9	F	0.6	111	15	0.15	c.2259delA	c.2259delA
4	W4	16	F	1.23	85	17	0.1	p.G550R	c.746_987 + 373del1782
5	YY	40	M	2.5	83	30.8	0.1	p.R973Nfs*14	p.G1031D

Serum creatinine (male) 0.5–1.1 mg/dl, (female) 0.4–0.8 mg/dl; Serum C3, 80–140 mg/dl; Serum C4, 10–40 mg/dl; Proteinuria, 0–30 mg/dl.

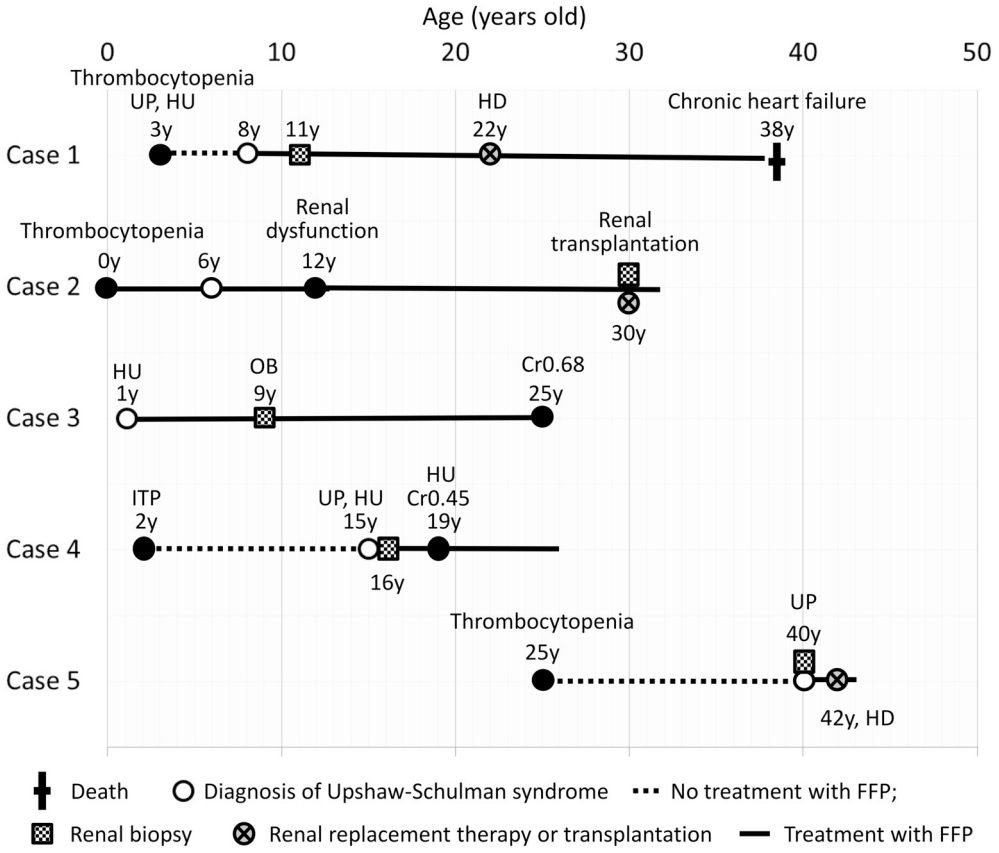

Fig. 1. Clinical courses of USS case 1–5. Cr, serum creatinine (mg/dl); FFP, fresh frozen plasma; HD, hemodialysis; HU, hematuria; ITP, idiopathic thrombocytopenic purpura; UP, proteinuria.

2.6. Statistical analyses

Categorical variables were compared using the Tukey honest significant difference test or Fisher exact test. JMP version 11 (SAS Institute, Cary, NC, USA) was used to perform Tukey honest significant difference tests and R version 3.2.5 to perform Fisher exact tests. A $p < 0.05$ (two-tailed) was considered statistically significant for all tests.

3. Results

3.1. Pathological findings

Pathological findings are summarized in Table 2. The majority of USS cases (four of five, cases 1, 2, 4, and 5) demonstrated chronic glomerular changes, while case 3 showed MGA. Chronic glomerular changes included collapsed capillaries with dilatation of Bowman's capsule (cases 1, 2, and 5), global or segmental sclerosis (cases 1, 2, 4, and 5), and duplication of basement membranes (case 4). In two cases [1 and 4], glomerular segmental sclerosis was predominantly localized in the perihilar region (3/3 glomeruli in case 1, 8/12 glomeruli in case 4). Interstitial fibrosis/tubular atrophy was severe in two cases [2 and 5] and mild in two cases [1 and 4]. In case 3, there was no tubulointerstitial scarring. Vascular changes were mild in most cases [1,3–5,and] and moderate in one (case 2), while one (case 5) exhibited intimal thickening and myxoid changes in the intima of the small arteries. No thrombus was observed in any case. Representative images of cases 1–5 are shown in Fig. 2A–F.

Previous immunofluorescence studies (IgA, IgG, IgM, and C3 staining) were negative in cases 4 and 5. Electron microscopy revealed a double contour of the glomerular basement membranes in case 4 (Fig. 2G) and subendothelial edema in case 5 (Fig. 2H). No electron-dense deposits were observed in any case. In cases 1, 2, and 3, results of previous immunofluorescence studies and electron microscopic findings were unavailable.

3.2. Immunohistological findings

To examine the relationships of these histolopathological manifestations with USS pathophysiology, we evaluated ADAMTS13, C4d, and C5b-9 expression levels and distribution in histological sections by immunohistological staining.

Table 2
Pathological characteristics of renal biopsies in USS.

Case no.	Sclerosed glomeruli/total glomeruli (%)	Segmental sclerosis	Duplication of the basement membranes	Thrombus	Myxoid intimal change of small arteries	IF/TA grade	Arteriosclerosis
1	11/17 (65)	+	−	−	−	I	Mild
2	30/35 (86)	+	−	−	−	III	Moderate
3	0/26 (0)	−	−	−	−	I	Mild
4	8/38 (21)	+	+	−	−	I	Mild
5	13/20 (65)	−	−	−	+	III	Mild

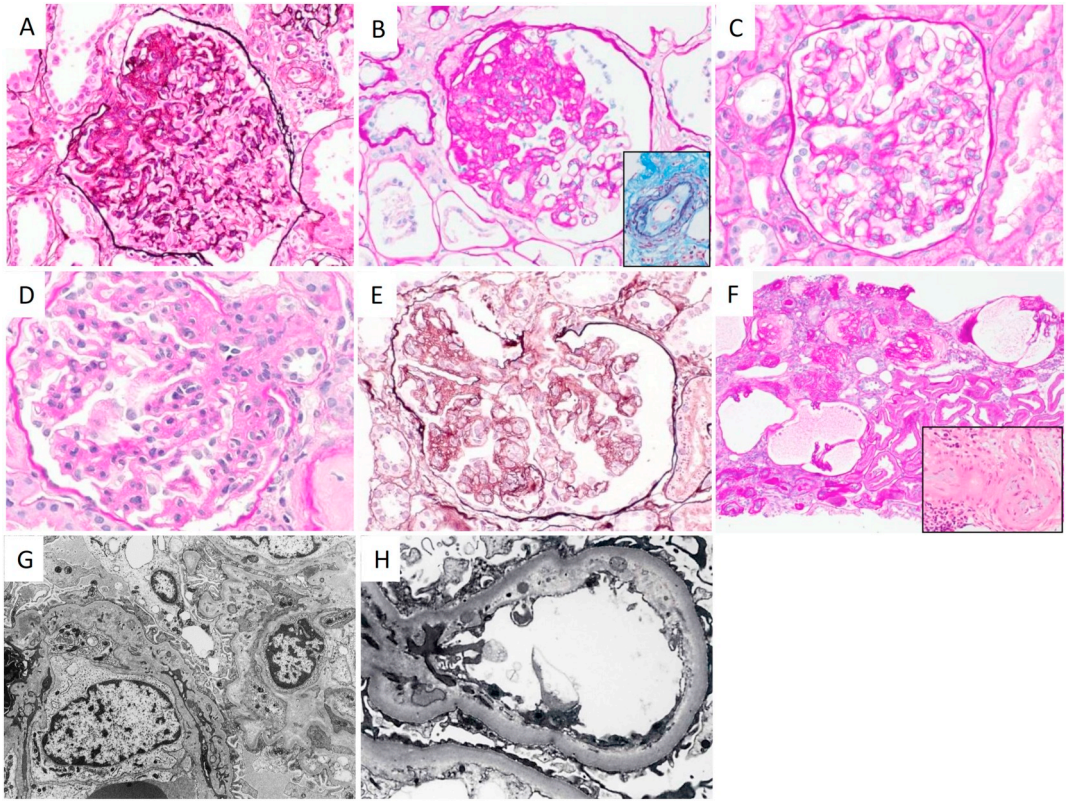

Fig. 2. (A)–(F) Light microscopic findings in the renal biopsies of (A) case 1, (B) case 2, (C) case 3, (D, E) case 4, and (F) case 5. (A) A glomerulus with segmental sclerosis localized in the perihilar region (PAM). (B) Glomerulus showing segmental sclerosis (PAS). A small artery exhibited intimal thickening without elastic fiber reduplication (inset, EMT). (C) Glomerulus with no significant pathological changes (PAS). (D) A glomerulus with segmental sclerosis. Glomerular tuft in the perihilar region shows solidification (PAS). (E) Glomerular capillaries showing diffuse double contour formation and focal segmental mesangial matrix increase (PAM). (F) Glomeruli showing global sclerosis and glomerular cyst (PAS). Small arteries showing myxoid intimal thickening (inset, HE). (G) and (H) Electron microscopic studies. (G) Double contour of the glomerular basement membranes (case 4). (H) Subendothelial edema in a glomerular capillary (case 5).

3.3. ADAMTS13

In control cases, glomerular ADAMTS13-positive cells were widely distributed (Fig. 3A). In contrast, glomeruli from USS and acquired TTP cases demonstrated sparsely distributed and weakly ADAMTS13-positive cells (Fig. 3B, C). The average number of ADAMTS13-positive cells per glomerulus was significantly lower in USS cases than controls (Fig. 4, $p = 0.0037$). The number of intraglomerular ADAMTS13-positive cells was also significantly lower in TTP samples compared to controls ($p = 0.0061$). There was no significant difference in the number of glomerular ADAMTS13-positive cells between USS and acquired TTP cases ($p = 0.7916$).

3.4. C4d

In control cases, C4d staining was detected mainly in the mesangium (Fig. 5A). In all USS patients except case 3 (with MGA), glomerular capillary walls were C4d-positive (Fig. 5B, C, D, E). Glomeruli were C4d-negative in case 3 (data not shown). In case 2, the luminal sides of small arteries were positive for C4d (Fig. 5C, inset). In acquired TTP cases, glomerular capillary walls were sparsely positive for C4d (Fig. 5F). The average number of C4d-positive capillaries per glomerulus was significantly higher in USS cases than controls (Fig. 6, $p = 0.0346$). The number of glomerular C4d-positive capillaries was also greater in acquired TTP cases than control cases, but the difference did not reach statistical significance ($p = 0.0686$). There was no significant difference in the number of glomerular C4d-positive capillaries between USS and acquired TTP cases ($p = 0.8086$). The number of C4d-positive small arteries did not differ significantly among the three groups (date not shown). Peritubular capillaries were C4d-negative in all cases.

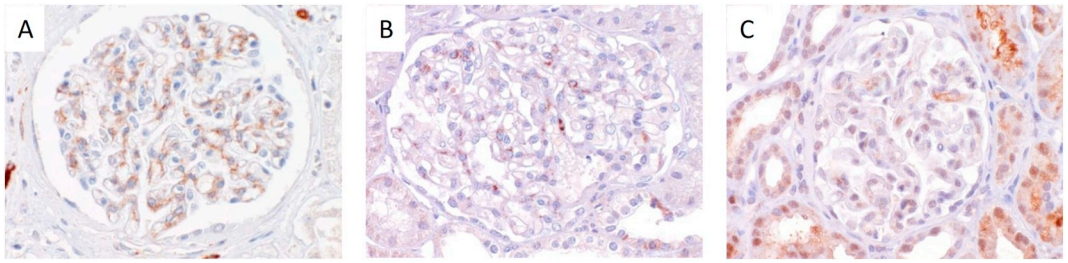

Fig. 3. Representative images of ADAMTS13 immunostaining in renal biopsy sections. (A) A glomerulus from a control case shows extensively distributed ADAMTS-13-positive cells. (B, C) A glomerulus from USS case 3 and an acquired TTP case (C) demonstrating sparsely distributed ADAMTS-13-positive cells within the glomerulus. Positive staining of renal tubules in (C) represents non-specific staining. Magnification × 400.

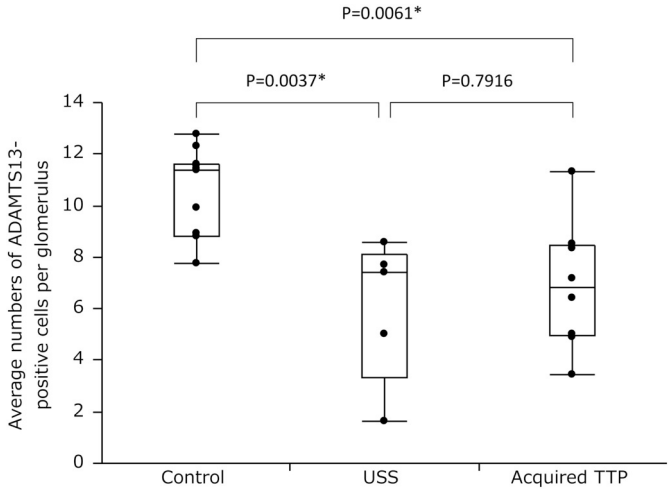

Fig. 4. Average numbers of ADAMTS13-positive cells per glomerulus among control, USS, and TTP groups. USS cases demonstrated fewer ADAMTS13-positive cells per glomerulus than control cases (p = 0.0037). Acquired TTP cases also showed fewer ADAMTS13-positive cells than control cases (p = 0.0061). There was no significant difference in the number of glomerular ADAMTS13-positive cells between USS and acquired TTP cases (p = 0.7916).

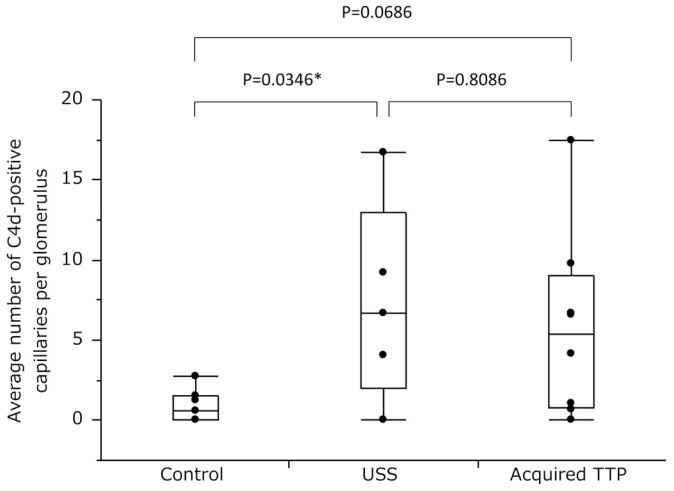

Fig. 6. Average number of C4d-positive capillaries per glomerulus among control, USS, and acquired TTP groups. USS cases demonstrated a greater number of C4d-positive capillaries per glomerulus than control cases (p = 0.0346). In acquired TTP cases, the number of glomerular C4d-positive capillaries was not statistically significant compared to either control cases (p = 0.0686) or USS cases (p = 0.8086).

3.5. C5b-9

Control cases exhibited C5b-9-positive staining in the vascular pole (Fig. 7A) or mesangium (data not shown). In USS cases, positive C5b-9 staining was observed along the glomerular capillary walls in cases 2 and 4 (Fig. 7C and D), whereas cases 1 (Fig. 7B), 3, and 5 showed no glomerular C5b-9 immunostaining. In two of ten acquired TTP cases, C5b-9 was sparsely positive along glomerular capillary walls (Fig. 7E). Luminal sides of the small arteries were positive for C5b-9 in three cases of USS (Fig. 7B, C, and D, insets) and four cases of acquired TTP (Fig. 7E). Peritubular capillaries were negative for C5b-9 in all groups. Quantitative examination revealed a higher average number of C5b-9-positive glomerular capillaries in USS cases compared to control cases and acquired TTP cases; however, the differences were not statistically significant (Fig. 8).

4. Discussion

Renal impairment is one of the major complications of USS, with acute kidney injury observed in 11% of cases [21] and progressive renal deterioration in approximately 50% of cases [22]. In the present study, three USS cases [1,2,5,and] developed chronic renal failure during the entire clinical course. Pathological examination of these cases revealed that more than half of all glomeruli examined showed sclerotic changes. Furthermore, cases 2 and 5 demonstrated severe IF/TA. Moderate sclerotic changes in the arteries were observed in case 2. In contrast, the two cases that did not progress to chronic renal failure (cases 3 and 4) demonstrated less severe glomerulosclerosis, IF/TA, and arteriosclerosis. Chronic lesions in glomeruli and tubulointerstitium are indicative of poor prognosis in chronic kidney diseases such as IgA nephropathy [23,24], lupus nephritis [25,26], and small vessel vasculitis [27–29]. Our study indicates that USS and other chronic kidney

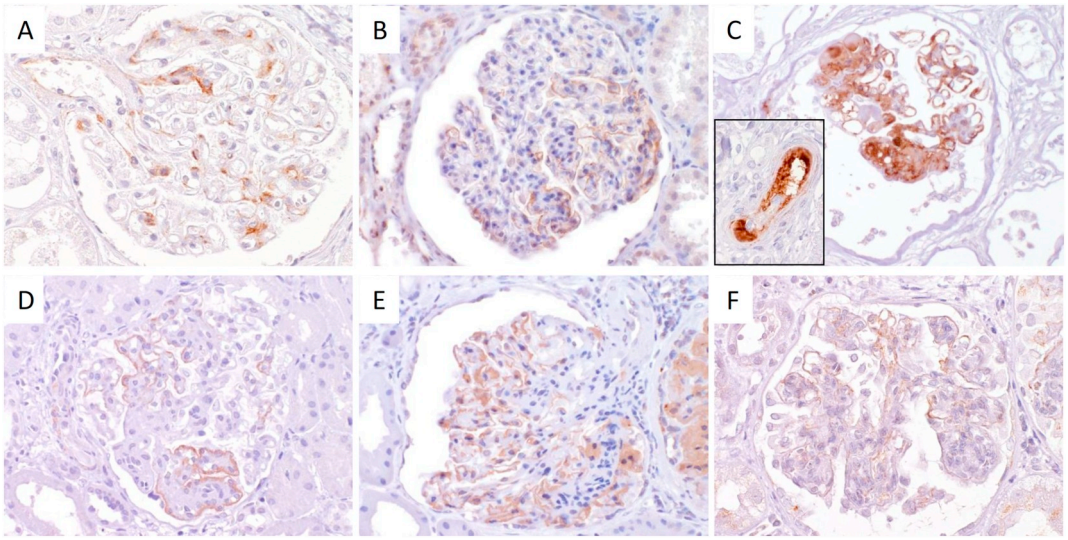

Fig. 5. Immunohistochemical staining for Cd4 in renal biopsy sections from (A) a control, (B) USS case 1, (C) case 2, (D) case 4, (E) case 5, and (F) an acquired TTP case. (A) In control cases, C4d was mainly positive in the mesangium. (B − E) In all USS patients except case 3 (not shown), C4d was immunopositive along the glomerular capillary walls. Case 2 demonstrated positive C4d staining in the vessel wall of small arteries (C, inset). (F) In acquired TTP cases, the glomerular capillary walls were positive for C4d. Magnification × 400.

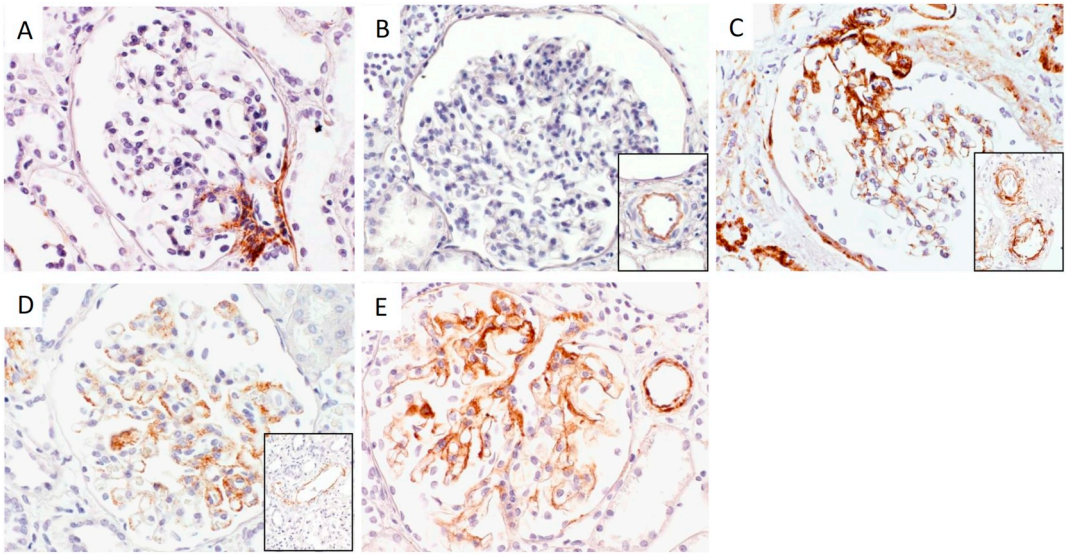

Fig. 7. Immunohistological staining for C5b-9 in renal biopsy sections from (A) a control case, (B) USS case 1, (C) case 2, (D) case 4, and (E) an acquired TTP case. (A) C5b-9 staining was limited to the vascular pole of the glomerulus. (B–D) C5b-9 staining was detected along the glomerular capillary walls in case 2 (C) and case 4 (D). In case 1 (B), case 2 (C), and case 4 (D), C5b-9 immunoreactivity was detected along the luminal side of arterioles (insets). (E) C5b-9 immunoreactivity was detected along the glomerular capillary walls and in arterioles. Magnification × 400.

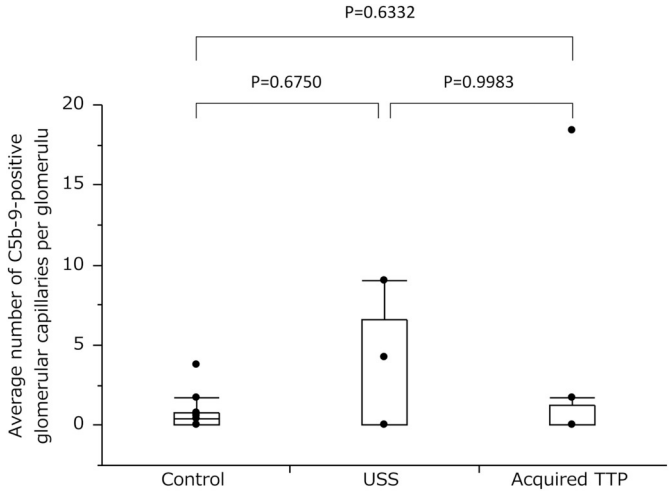

Fig. 8. Average numbers of C5b-9-positive capillaries per glomerulus in control, USS, and acquired TTP groups. Number of glomerular C5b-9-positive capillaries did not differ significantly among the three groups.

diseases share common histological features that are associated with poor renal outcome. Further, our immunohistochemical analyses implicate complement activation associated with ADAMTS13 deficiency in USS pathogenesis.

Immunohistological examinations in our study highlighted the pathophysiological features of these characteristic histological manifestations in USS. In human renal tissue, podocytes, glomerular endothelial cells, and renal tubular cells produce ADAMTS13 [9–11]. Podocyte-derived ADAMTS13 contributes to protection against shear stress in the glomerular microcirculation [21]. Therefore, reduced numbers of intraglomerular ADAMTS13-positive cells in USS may render glomerular endothelial cells more susceptible to hemodynamic injury. Indeed, the predominant localization of segmental sclerosis in the glomerular hilum of the USS cases in our study suggests a causal relationship between ADAMTS13 deficiency and hemodynamic injury. In the glomerular hilum, afferent and efferent arterioles as well as branching glomerular capillaries create complex directions of blood flow [30], causing high shear stress in endothelial cells. Three-dimensional reconstruction of the glomerulus and theoretical model analysis revealed higher ultrafiltration pressure in glomerular capillaries near the afferent arteriole [31]. A recent study also found an association between intraglomerular hypertension and focal segmental glomerulosclerosis with perihilar lesions [32]. Thus, enhanced vulnerability of the hilum in USS may reflect generally higher blood flow combined with reduced ADAMTS13-dependent protection against shear stress.

Two cases in USS showed severely reduced numbers of ADAMTS13-positive cells within the glomerulus. In contrast, three cases showed less severe decrease in the number of ADAMTS13-positive cells. Pattern of *ADAMTS13* gene mutations in Table 1 did not correlate the immunohistological ADAMTS13 staining, suggesting non-genetic mechanism affected ADAMTS13 expression. Likewise, acquired TTP cases in our study had significantly reduced glomerular ADAMTS13 positivity compared to normal controls. This results can be partly attributed to two cases of systemic lupus erythematosus which showed particularly lower number (< 5 cells) of ADAMTS13-positive cells in the glomeruli. In systemic lupus erythematosus, severely reduced ADAMTS13 activity in the presence of non-neutralizing IgG anti-ADAMTS13 antibodies have been reported [33], suggesting that rare cases of secondary TTP may be associated with mechanisms independent of the direct inhibition of ADAMTS13 by autoantibodies; increased clearance of the protease-antibody complex or interference with protease binding to the cell surface can be one potential cause [34]. Another possible explanation includes *ADAMTS13* gene mutation causing major structural alteration of ADAMTS13 protein, which may be associated with decreased activity of ADAMTS13 and result in decreased ADAMTS13 immunostaining of glomeruli; however, we did not perform *ADAMTS13* gene mutation analysis in acquired TTP cases, rendering mechanistic insight of decreased ADAMTS13 immunostaining in acquired TTP cases inconclusive.

Complement protein C4d has been utilized as an immunohistological marker for complement activation in the antibody-mediated rejection of renal allografts [35] and for TMA, even in clinically heterogeneous patient populations [13,36]. Our findings of glomerular C4d deposition in USS cases are consistent with a previous study demonstrating glomerular complement activation by immunostaining for C3 and C5b-9 in patients with *ADAMTS13* mutations [14]. In addition, prompt disease remission after treatment with the

anti-C5 monoclonal antibody eculizumab [37] further supports the involvement of complement activation in the pathogenesis of USS. However, the numbers of C5b-9-positive capillaries did not differ significantly between USS cases and controls in our study, suggesting that C5b-9 immunoreactivity is an unsuitable ancillary marker of complement activation in USS patients.

In contrast to previous studies demonstrating acute-type thrombotic microangiopathy in renal biopsies of USS patients, including fibrin thrombi and endothelial cell proliferation [9], our study demonstrated chronic glomerular changes in all but one case. Cases 1, 2, and 3 had received infusion of FFP from 3 to 30 years prior to renal biopsy, while renal biopsies were not preceded by FFP in cases 4 and 5. Although effective in preventing acute episodes of thrombocytopenia and hemolytic anemia [38], it is still unclear whether prophylactic FFP infusion therapy can prevent the progressive decline of renal function in USS [21,39], and there is currently no standard treatment strategy for USS patients in remission.

ADAMTS13 gene mutations are not always sufficient to cause acute clinical presentation in USS. In our study, case 2 harbored the *ADAMTS13* gene mutation p.C908Y (maternal origin) also detected in a previous report of a homozygous USS patient that progressed to end-stage kidney disease [5]. In the same study, however, four other patients harboring the p.C908Y mutation did not progress to dialysis during follow-up. In a recent report by Pecoraro et al., a USS patient harboring two heterozygous mutations (a guanine to adenine mutation at nucleotide 3251 and a novel frameshift after arginine-1351, resulting in a premature stop codon nine amino acids upstream) experienced acute episodes of hematologic relapse following upper respiratory tract infection [37]. Thus, in addition to genetic mutations, other disease-triggering factors such as infection, alcohol intake, pregnancy, trauma, and surgery may contribute to disease onset in USS [5,6,40–42].

Our study has several limitations. The small sample size precludes a quantitative description of the relationship between immunohistological findings and renal outcome. Therefore, further studies are needed in more numbers of USS cases to warrant the conclusion. Also, immunostaining tends to be less sensitive in paraffin sections compared to frozen sections [35]. Therefore, future studies should investigate glomerular C4d and C5b-9 distributions in frozen sections from biopsy samples.

In summary, this study of five USS cases demonstrated chronic glomerular microangiopathic changes accompanied by decreased expression of ADAMTS13-positive cells and a greater average number of C4d-positive capillaries compared to controls. Glomerular C4d positivity in USS may be useful as a marker for complement activation. The present results warrant further prospective studies that focus on the association of renal outcome and C4d immunostaining in USS patients.

4.1. Compliance with ethical standards

The study protocol was approved by the Ethics Committee of Nara Medical University and conducted in accordance with the tenets of the Declaration of Helsinki.

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No funding was received.

Conflict of interest

The authors declare that they have no conflict of interest.

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References

[1] G.G. Levy, W.C. Nichols, E.C. Lian, T. Foroud, J.N. McClintick, B.M. McGee, et al., Mutations in a member of the ADAMTS gene family cause thrombotic thrombocytopenic purpura, Nature 413 (2001) 488–494.
[2] V. Bianchi, R. Robles, L. Alberio, M. Furlan, B. Lammle, Von willebrand factor-cleaving protease (ADAMTS13) in thrombocytopenic disorders: a severely deficient activity is specific for thrombotic thrombocytopenic purpura, Blood 100 (2002) 710–713.
[3] X.L. Zheng, H.M. Wu, D. Shang, E. Falls, C.G. Skipwith, S.R. Cataland, et al., Multiple domains of ADAMTS13 are targeted by autoantibodies against ADAMTS13 in patients with acquired idiopathic thrombotic thrombocytopenic purpura, Haematologica 95 (2010) 1555–1562.
[4] K. Kokame, M. Matsumoto, K. Soejima, H. Yagi, H. Ishizashi, M. Funato, et al., Mutations and common polymorphisms in ADAMTS13 gene responsible for von Willebrand factor-cleaving protease activity, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 11902–11907.
[5] Y. Fujimura, M. Matsumoto, A. Isonishi, H. Yagi, K. Kokame, K. Soejima, et al., Natural history of Upshaw-Schulman syndrome based on ADAMTS 13 gene analysis in Japan, J. Thromb. Haemost. 9 (2011) 283–301.
[6] M. Moatti-Cohen, C. Garrec, M. Wolf, P. Boisseau, L. Galicier, E. Azoulay, et al., Unexpected frequency of Upshaw-Schulman syndrome in pregnancy-onset thrombotic thrombocytopenic purpura, Blood 119 (2012) 5888–5897.
[7] M. Uemura, K. Tatsumi, M. Matsumoto, M. Fujimoto, T. Matsuyama, M. Ishikawa, et al., Localization of ADAMTS13 to the stellate cells of human liver, Blood 106 (2005) 922–924.
[8] W. Zhou, M. Inada, T.P. Lee, D. Benten, S. Lyubsky, E.E. Bouhassira, et al., ADAMTS13 is expressed in hepatic stellate cells, Lab. Investig. 85 (2005) 780–788.
[9] M. Manea, A. Kristoffersson, R. Schneppenheim, M.A. Saleem, P.W. Mathieson, M. Mörgelin, et al., Podocytes express ADAMTS13 in normal renal cortex and in patients with thrombotic thrombocytopenic purpura, Br. J. Haematol. 138 (2007) 651–662.
[10] M. Manea, R. Tati, J. Karlsson, Z.D. Békássy, D. Karpman, Biologically active ADAMTS13 is expressed in renal tubular epithelial cells, Pediatr. Nephrol. 25 (2010) 85–96.
[11] R. Tati, A.C. Kristoffersson, A.L. Ståhl, M. Mörgelin, D. Motto, S. Satchel, et al., Phenotypic expression of ADAMTS13 in glomerular endothelial cells, PLoS One 6 (2011) e21587.
[12] N. Kambham, Thrombotic microangiopathies, in: R.B. Colvin, A. Chang, A.B. Farris, 3rdN. Kambham, L.D. Cornell, S.M. Meehan, et al. (Eds.), Diagnostic Pathology: Kidney Diseases, second ed., Elsevier, Philadelphia, PA, 2015, pp. 518–549.
[13] J.S. Chua, H.J. Baelde, M. Zandbergen, S. Wilhelmus, L.A. van Es, J.W. de Fijter, et al., Complement factor C4d is a common denominator in thrombotic microangiopathy, J. Am. Soc. Nephrol. 26 (2015) 2239–2247.
[14] R. Tati, A.C. Kristoffersson, A.L. Ståhl, J. Rebetz, L. Wang, C. Licht, et al., Complement activation associated with ADAMTS13 deficiency in human and murine thrombotic microangiopathy, J. Immunol. 191 (2013) 2184–2193.
[15] M. Miura, S. Koizumi, K. Nakamura, T. Ohno, T. Tachinami, M. Yamagami, et al., Efficacy of several plasma components in a young boy with chronic thrombocytopenia and hemolytic anemia who responds repeatedly to normal plasma infusions, Am. J. Hematol. 17 (1984) 307–319.
[16] M. Matsumoto, K. Kokame, K. Soejima, M. Miura, S. Hayashi, Y. Fujii, et al., Molecular characterization of ADAMTS13 gene mutations in Japanese patients with Upshaw-Schulman syndrome, Blood 103 (2004) 1305–1310.
[17] H. Saitoh, H. Murakami, C. Mori, Upshaw-Schulman syndrome in two siblings, Acta Paediatr. Jpn. 32 (1990) 373–376.
[18] T. Doi, S. Ohga, N. Ito, M. Ishimura, N. Suga, A. Nomura, et al., Limited renal prophylaxis in regular plasmatherapy for heritable ADAMTS13 deficiency, Pediatr. Blood Cancer 60 (2013) 1557–1558.
[19] K. Solez, R.B. Colvin, L.C. Racusen, M. Haas, B. Sis, M. Mengel, et al., Banff 07 classification of renal allograft pathology: updates and future directions, Am. J. Transplant. 8 (2008) 753–760.
[20] C.L. Bockmeyer, V. Forstmeier, F. Modde, S. Lovric, R.A. Claus, M. Schiffer, et al., ADAMTS13-marker of contractile phenotype of arterial smooth muscle cells lost in benign nephrosclerosis, Nephrol. Dial. Transplant. 26 (2011) 1871–1881.
[21] H.M. Tsai, The kidney in thrombotic thrombocytopenic purpura, Minerva Med. 98 (2007) 731–747.
[22] B.M. John, D. Singh, B. Ravichander, R. Madan, T.S. Raghu Raman, Upshaw-Schulman syndrome, Med. J. Armed Forces India 66 (2010) 188–189.
[23] R. Coppo, S. Troyanov, S. Bellur, D. Cattran, H.T. Cook, J. Feehally, et al., Validation of the Oxford classification of IgA nephropathy in cohorts with different presentations and treatments, Kidney Int. 86 (2014) 828–836.
[24] K. El Karoui, G.S. Hill, A. Karras, C. Jacquot, L. Moulonguet, O. Kourilsky, et al., A clinicopathologic study of thrombotic microangiopathy in IgA nephropathy, J. Am. Soc. Nephrol. 23 (2012) 137–148.
[25] H.A. Austin 3rd, D.T. Boumpas, E.M. Vaughan, J.E. Balow, Predicting renal outcomes in severe lupus nephritis: contributions of clinical and histologic data, Kidney Int. 45 (1994) 544–550.
[26] N. Hiramatsu, T. Kuroiwa, H. Ikeuchi, A. Maeshima, Y. Kaneko, K. Hiromura, et al., Revised classification of lupus nephritis is valuable in predicting renal outcome with an indication of the proportion of glomeruli affected by chronic lesions,

Rheumatology 47 (2008) 702–707.
[27] A.E. Berden, F. Ferrario, E.C. Hagen, D.R. Jayne, J.C. Jennette, K. Joh, et al., Histopathologic classification of ANCA-associated glomerulonephritis, J. Am. Soc. Nephrol. 21 (2010) 1628–1636.
[28] D.Y. Chang, L.H. Wu, G. Liu, M. Chen, C.G. Kallenberg, M.H. Zhao, Re-evaluation of the histopathologic classification of ANCA-associated glomerulonephritis: a study of 121 patients in a single center, Nephrol. Dial. Transplant. 27 (2012) 2343–2349.
[29] E. Muso, T. Endo, M. Itabashi, H. Kakita, Y. Iwasaki, Y. Tateishi, et al., Evaluation of the newly proposed simplified histological classification in Japanese cohorts of myeloperoxidase-anti-neutrophil cytoplasmic antibody-associated glomerulonephritis in comparison with other Asian and European cohorts, Clin. Exp. Nephrol. 17 (2013) 659–662.
[30] K.M. Newhold, A.J. Howie, Analysis of the position of segmental lesions in glomeruliin vasculitic-type glomerulonephritis and other disorders, J. Pathol. 162 (1990) 149–155.
[31] A. Remuzzi, B.M. Brenner, V. Pata, G. Tebaldi, R. Mariano, A. Belloro, et al., Three-dimensional reconstructed glomerular capillary network: blood flow distribution and local filtration, Am. J. Phys. 263 (1992) F562–F572.
[32] V.D. D'Agati, A.B. Fogo, J.A. Bruijn, J.C. Jennette, Pathologic classification of focal segmental glomerulosclerosis: a working proposal, Am. J. Kidney Dis. 43 (2004) 368–382.
[33] M.E. Knecht, M. Mayr, S. Ferrari, F. Scheiflinger, M. Trendelenburg, A patient with SLE-associated thrombotic microangiopathy and non-neutralizing antibodies against ADAMTS13, Nephrol. Dial. Transplant. 25 (2010) 1720–1722.
[34] F. Scheiflinger, P. Knöbl, B. Trattner, B. Plaimauer, G. Mohr, M. Dockal, et al., Nonneutralizing IgM and IgG antibodies to von Willebrand factor-cleaving protease (ADAMTS-13) in a patient with thrombotic thrombocytopenic purpura, Blood 102 (2003) 3241–3243.
[35] K. Solez, R.B. Colvin, L.C. Racusen, M. Haas, B. Sis, M. Mengel, et al., Banff 07 classification of renal allograft pathology: updates and future directions, Am. J. Transplant. 8 (2008) 753–760.
[36] A.H. Gasim, J.S. Chua, R. Wolterbeek, J. Schmitz, E. Weimer, H.K. Singh, et al., Glomerular C4d deposits can mark structural capillary wall remodeling in thrombotic microangiopathy and transplant glomerulopathy: C4d beyond active antibody mediated injury, Transpl. Int. 30 (2017) 519–532.
[37] C. Pecoraro, A.V. Ferretti, E. Rurali, M. Galbusera, M. Noris, G. Remuzzi, Treatment of congenital thrombotic thrombocytopenic purpura with eculizumab, Am. J. Kidney Dis. 66 (2015) 1067–1070.
[38] J.A. Kremer Hovinga, P. Coppo, B. Lämmle, J.L. Moake, T. Miyata, K. Vanhoorelbeke, Thrombotic thrombocytopenic purpura, Nat. Rev. Dis. Prim. 3 (2017) 17020.
[39] P. Blombery, M. Scully, Management of thrombotic thrombocytopenic purpura: current perspectives, J. Blood Med. 5 (2014) 15–23.
[40] M. Morioka, M. Matsumoto, M. Saito, K. Kokame, T. Miyata, Y. Fujimura, A first bout of thrombotic thrombocytopenic purpura triggered by herpes simplex infection in a 45-year-old nulliparous female with Upshaw-Schulman syndrome, Blood Transf. (2014) s153–s155.
[41] T. Falter, J.A. Kremer Hovinga, K. Lackner, H.G. Füllemann, B. Lämmle, I. Scharrer, Late onset and pregnancy-induced congenital thrombotic thrombocytopenic purpura, Hamostaseologie 34 (2014) 244–248.
[42] N. Epperla, K. Hemauer, K.D. Friedman, J.N. George, P. Foy, Congenital thrombotic thrombocytopenic purpura related to a novel mutation in ADAMTS13 gene and management during pregnancy, Am. J. Hematol. 91 (2016) 644–646.