【 CASE REPORT 】

A Rare Case of Lupus Nephritis Presenting as Thrombotic Microangiopathy with Diffuse Pseudotubulization Possibly Caused by Atypical Hemolytic Uremic Syndrome

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Abstract:
A 31-year-old woman was admitted to our hospital for thrombotic microangiopathy (TMA). She was diagnosed with systemic lupus erythematosus (SLE) and class V lupus nephritis. She had no aggravated SLE activity, Shiga toxin positivity, ADAMTS13 abnormality, or other causes of secondary TMA. Plasma exchange partially improved TMA, and eculizumab was introduced for suspected atypical hemolytic uremic syndrome (aHUS), as eculizumab was effective in suppressing the TMA activity. A kidney biopsy revealed diffusely organized crescents (pseudotubulization) with glomerular and arteriolar endothelial injury and subepithelial immune deposits. Thus, this was a rare case of lupus nephritis presenting as TMA with pseudotubulization possibly caused by aHUS.

Key words: thrombotic microangiopathy, systemic lupus erythematosus, atypical hemolytic uremic syndrome, eculizumab, pseudotubulization

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Introduction

The kidney is prone to injury from thrombotic microangiopathy (TMA), which is clinically defined as a condition showing 1) hemolytic anemia, 2) thrombocytopenia, and 3) organ injuries due to microvascular thrombi. The etiologies of TMA are classified into four categories: Shiga toxin-producing *Escherichia coli*-mediated hemolytic uremic syndrome (STEC-HUS), thrombotic thrombocytopenic purpura (TTP), complement-mediated atypical hemolytic uremic syndrome (aHUS), and secondary TMA (1).

The involvement of TMA in the kidney is characterized by thickening of the capillary walls accompanied by a double-contoured appearance, fragmented red blood cells, fibrin, and platelet thrombi in the capillary lumens and focal mesangiolysis in the glomeruli. A narrowed arteriolar lumen, fragmented red blood cells in the thickened arteriolar walls, and infiltration of the arteriolar walls by fibrin are also detected in the arterioles (2).

It has been reported that pseudotubulization is caused by the organization of crescent formation, which is rarely complicated by TMA (3-5). We herein report a rare case of lupus nephritis presenting as diffusely organized crescents (pseudotubulization) after treatment with eculizumab for TMA possibly caused by aHUS but not by lupus nephritis.

Case Report

A 31-year-old woman was admitted to our hospital for hemolytic anemia, thrombocytopenia, and acute kidney injury (AKI). She had experienced repeated episodes of severe

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pneumonia since childhood. She was diagnosed with systemic lupus erythematosus (SLE) based on lymphocytopenia, proteinuria greater than 0.5 g/day, pleuritis, pericarditis, and positive findings for antinuclear and anti-Smith antibodies according to the American College of Rheumatology criteria in a previous hospital approximately 7 months before admission to our hospital. A urinalysis revealed proteinuria (urinary protein: 3-4+ and daily urinary protein 1-2 g) without apparent hematuria. A kidney biopsy was performed, and she was diagnosed with class V lupus nephritis because of thickening and spike formation of the capillary walls and positive granular staining for IgG and C1q in the capillaries of the glomerulus.

Over the course of 1 month, beginning 2 months prior to admission to our hospital, her serum creatinine (sCr) had increased from 0.44 mg/dL to 0.78 mg/dL accompanied by exacerbation of pleuritis and pericarditis without changes in the urinalysis results. Prednisolone (PSL) was increased from 12.5 mg/day to 15 mg/day, and mizoribine 150 mg/day was initiated 5 weeks before admission, which improved her serositis but not the renal dysfunction. Her sCr increased to 1.13 mg/dL 10 days before hospitalization, when hemolytic anemia and thrombocytopenia concurred. She was transferred to our hospital for a further evaluation.

On admission, her blood pressure was 138/74 mmHg, pulse rate was 58 beats/min, she was afebrile, and other vital signs and physical examination findings were also unremarkable. The laboratory data at admission are shown in Table. Her hemoglobin was 7.4 g/dL, reticulocyte ratio 2.4%, haptoglobin below the laboratory sensitivity, and lactate dehydrogenase (LDH) 392 IU/L, indicating hemolytic anemia. Thrombocytopenia was also found (platelets: 77,000/μL). Her aberrant renal function was further exacerbated after admission (sCr: 2.65 mg/dL and estimated glomerular filtration rate: 18 mL/min/1.73 m^2). Serological tests revealed negativity for anti-double-stranded DNA antibody. Hypocomplementemia was remarkable but did not differ from the previous results. In addition, no signs suggestive of other collagen diseases, including anti-phospholipid antibody syndrome (APS) and scleroderma, were found. A urinalysis revealed proteinuria (urinary protein: 3+, and 1,855 mg/day) but no apparent hematuria.

The clinical course of the present case is shown in Fig. 1. We administered methylprednisolone pulse therapy at 0.5 g/day for 3 days followed by oral PSL at 50 mg/day. However, hemolytic anemia, thrombocytopenia, and renal failure deteriorated with fragmented erythrocytes, indicating the development of TMA. Plasma exchange was started on the 15th day, and hemodialysis was also initiated. Plasma exchange improved the activity of TMA temporarily, but we had to continue plasma exchange to maintain appropriate levels of hemoglobin and platelets.

TMA developed without diarrhea or a low activity of ADAMTS13. In addition, TMA was exacerbated independent of the SLE activity. No signs suggestive of other causes of TMA, such as APS, infections, malignancy, or drugs, were found. Therefore, we strongly suspected that the etiology of TMA might be aHUS, although the anti-CFH antibody was negative before plasma exchange. To discontinue plasma exchange, the C5 monoclonal antibody eculizumab was initiated from the 52nd day of admission (introductory dose: 900 mg by 1 week; and maintenance dose: 1,200 mg by 2 weeks). Plasma exchange was able to be replaced by treatment with eculizumab provided the levels of hemoglobin and platelet were controlled. However, hemodialysis could not be discontinued.

A kidney biopsy was performed three months after admission. The tissue contained 22 glomeruli. Global sclerosis was not found in the glomeruli. There were adhesions between the Bowman's capsule and the glomerular tuft in five glomeruli. Organized crescents (pseudotubulization) were found in 13 glomeruli. Most of the glomeruli demonstrated a tendency to collapse and had wrinkling changes in the capillary walls and luminal narrowing (Fig. 2A and B). In addition, thickening, spike formation, a bubbly appearance, and double-contouring of the capillary walls were detected in most glomeruli (Fig. 2C). Diffuse interstitial fibrosis and tubular atrophy were found in approximately 50% of tubulointerstitial areas (Fig. 2D). Although schistocytes and fibrin formation were not found, swelling of the endothelial cells and luminal narrowing and obstruction were detected in many arterioles and small arteries (Fig. 2E and F). Immunofluorescent findings revealed slightly positive staining for IgG in the capillaries and part of the mesangial region of the glomerulus (Fig. 2G). Other immunoreactants, including C1q, were negative.

Electron microscopy revealed thickening of the glomerular basement membrane, partial effacement of the foot process (Fig. 3), and swelling of endothelial cells (Fig. 3B). There were slightly enlarged subendothelial spaces (Fig. 3A) and mesangial interpositions (Fig. 3B) with a newly synthesized basement membrane, forming a double contour. Electron-dense deposits (EDDs) of various sizes were seen in the subepithelial and intramembranous regions (Fig. 3A), and some EDDs were also found in some mesangial regions. Given these results, the kidney biopsy revealed that the endothelial injuries of glomerular capillaries, arterioles, and small arteries, suggestive of TMA, were complicated with class V lupus nephritis. In addition, pseudotubulization had developed in many of glomeruli, possibly associated with TMA.

The patient was discharged three months after starting eculizumab. Her renal function did not ameliorate at all, and hemodialysis could not be discontinued despite continued eculizumab therapy. However, her hemoglobin and platelet levels improved and remained stable. Therefore, we judged that although the renal damage was irreversible, her acute endothelial injury was improved by eculizumab therapy. Therefore, the therapy was withdrawn nine months after starting eculizumab, and aggravation of aHUS was not observed.

Table.   **Laboratory Data on Admission.**

Hematology		Biochemistry				
WBC	4,190 /μL	Na	145 mEq/L	LDH	392 IU/L	
neutro	89 %	K	4.4 mEq/L	GOT	16 IU/L	
eosino	0 %	Cl	113 mEq/L	GPT	11 IU/L	
baso	0 %	Ca	7.7 mg/dL	CPK	42 IU/L	
lympho	8 %	P	4.2 mg/dL	ALP	160 IU/L	
mono	3 %	Mg	1.7 mg/dL	γGTP	35 IU/L	
RBC	216×10^4 /μL			ChE	241 mg/dL	
Hb	7.4 g/dlL	UA	10.4 mg/dL			
Ht	21.6 %	BUN	60.4 mg/dL	TG	139 μg/dL	
MCV	101 FL	Cre	2.65 mg/dL	T-Chol	195 ng/mL	
MCH	34.3 Pg	eGFR	18 mL/min/1.73m^2	LDL-C	133 mg/dL	
MCHC	33.8 %					
Plt	7.7×10^4 /μL	TP	4.7 g/dL	Fe	86 g/dL	
Ret	2.4 %	Alb	2.6 g/dL	TIBC	220 g/dL	
		T.Bil	0.6 mg/dL	Ferritin	486 μg/dL	
		D.Bil	<0.1 mg/dL			
				Haptoglobin	<10 mg/dL	

Serology				
CRP	0.02 mg/dL	MPO-ANCA	<1.0 U/mL	
IgG	610 mg/dL	PR3-ANCA	<1.0 U/mL	
IgA	130 mg/dL	Anti-GBM Ab	<2.0 U/mL	
IgM	327 mg/dL	Direct coombs test	negative	
ANA	1/320	Anti-platelet Ab	negative	
speckled	1/320	Anti-cardiolipin Ab	<8	
Anti-ds-DNA Ab	<10 U/mL	Anti-CL·β2GP1 Ab	<1.2 U/mL	
Anti-RNP Ab	1/4	Lupus anticoagulant	1.09 U/mL	
Anti-SSA Ab	1/1			
Anti-SSB Ab	(-)	ADAMTS13 activity	83 %	
C3	22 mg/dL			
C4	4 mg/dL	Coagulation system		
CH50	11 U/mL	PT	>140 %	
C1q	<1.5 μg/mL	APTT	107 %	
		Fib	331 mg/dL	
		D-dimer	2.3 μg/mL	

Urinalysis				
pH	6.0	Protein excretion	1,855 mg/day	
Protein	3+	Creatinine excretion	304.3 mg/day	
Glucose	-			
Ketone	-	Urinary assays		
Urobilinogen	±	β2MG	21,925 μg/L	
Bilirubin	-	α1MG	79.9 mg/L	
Occult blood	1+	NAG	35.7 U/L	
Nitrituria	-			
Specific gravity	1.017			
Red blood cell	3-5 /hpf			
White blood cell	1-3 /hpf			

gGTP: gamma glutamyltransferase, Ab: antibody, Alb: albumin, ALP: alkaline phosphatase, ANA: antinuclear antibody, APTT: activated partial thromboplastin time, BUN: blood urea nitrogen, CH50: total complement activity, ChE: cholinesterase, Clq: complement 1q, CPK: creatinine phosphokinase, Cre: creatinine, CRP: C-reactive protein, D.Bil: direct bilirubin, eGFR: estimated glomerular filtration rate, GBM: glomerular basement membrane, AST: aspartate transaminase, ALT: alanine transaminase, glutamate pyruvate transaminase, Hb: hemoglobin, Ht: hematocrit, Ig: immunoglobulin, LDH: lactate dehydrogenase, LDL-C: low-density lipoprotein cholesterol, MCH: mean cell hemoglobin, MCHC: mean cell hemoglobin concentration, MCV: mean corpuscular volume, MPO-ANCA: myeloperoxidase anti-neutrophil cytoplasmic antibodies, Plt: platelets, PR3-ANCA: proteinase-3 anti-neutrophil cytoplasmic antibodies, PT: prothrombin time, RBC: red blood cells, Ret: reticulocytes, RNP: ribonucleoprotein, T.Bil: total bilirubin, T-Chol: total cholesterol, TG: triglycerides, TIBC: total iron-binding capacity, TP: total protein, UA: uric acid, WBC: white blood cell
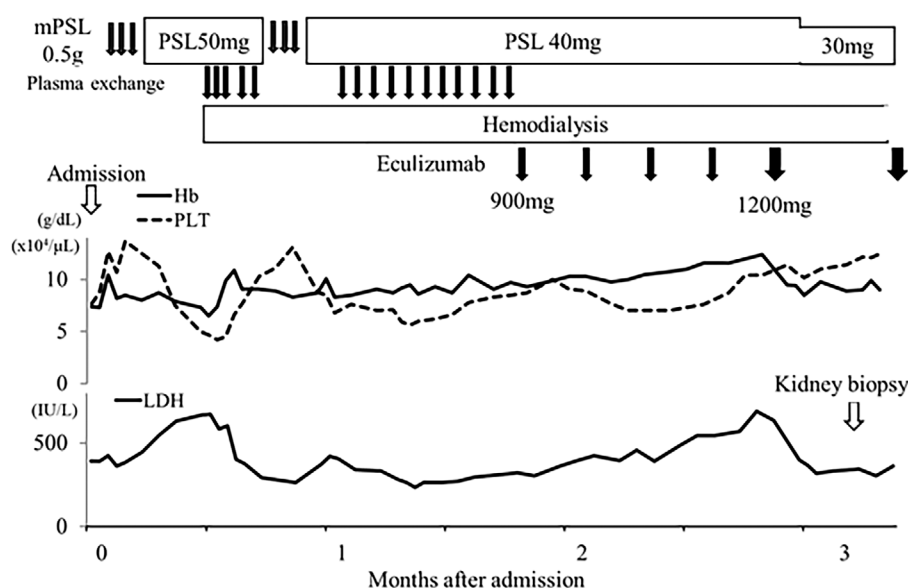
Figure 1. Clinical course after admission in the present case. Hb: hemoglobin, LDH: lactate dehydrogenase, mPSL: methylprednisolone pulse, PLT: platelets, PSL: prednisolone

Discussion

We reported a rare case of lupus nephritis showing organized crescents (pseudotubulization) due to TMA possibly caused by aHUS. The pseudotubulization was thought to have been formed as a result of cellular crescent organization (6). However, it is unlikely that the diffuse pseudotubulization in our patient was caused by lupus nephritis, due to the lack of aggravated SLE activity and the presence of glomerular endothelial damage with double-contouring of the capillary walls without subendothelial immune deposits.

It has been reported that crescent formation is rarely associated with renal TMA; indeed, to our knowledge, only three cases have been described (3-5). Although the mechanisms underlying crescent formation in renal TMA have not been clarified, we hypothesized that endothelial injury spread outside the capillary across the glomerular basement membrane, leading to extracapillary proliferation, when the activity of TMA was severe, similar to the interaction between endothelial injury and crescent formation in the pauci-immune type of anti-neutrophil cytoplasmic antibody-associated glomerulonephritis, anti-glomerular basement membrane glomerulonephritis, and the immune-complex type of glomerulonephritis (7). Interestingly, all crescent formations in our case showed a similar organized phase, and pseudotubulization was seen in 60% of glomeruli. These findings suggest that most glomeruli were attacked by TMA in a relatively short time. In the present case, diffuse cellular crescents might have been formed in response to massive glomerular endothelial injury with rupture of the capillary walls that was eventually partly resolved by aggressive therapy, including a high dose of corticosteroids, plasma exchange, and eculizumab, resulting in diffuse pseudotubular formations.

TMA in patients with SLE can be caused by its complications, such as class III/IV lupus nephritis, anti-phospholipid antibody syndrome (APS), thrombotic thrombocytopenic purpura (TTP), scleroderma, malignant hypertension, and adverse effects of calcineurin inhibitors (8). However, in the present case, no signs suggestive of these complications, including APS, were noted. It is difficult to determine the etiology of SLE-related TMA without complications of SLE, such as APS, although the activation of the classical pathway of the complement might be involved in the development of TMA in lupus nephritis (9). Di Song reported that 24% of lupus nephritis cases were accompanied by renal TMA lesions (8). These renal TMA lesions were rarely accompanied by class V lupus nephritis without other complications (8). Therefore, the absence of subendothelial immune complex formation suggested that the renal TMA in our case was caused by a non-lupus nephritis origin. Although the activity of SLE was controlled on admission in this case, the renal function further decreased in association with TMA despite the administration of high-dose corticosteroids and plasma exchange. The involvement of aHUS should be suspected when TMA continues after extensive treatment of the primary diseases with secondary TMA (10-13). It has also been reported that complement-amplifying conditions, such as primary diseases with secondary TMA, including infections, SLE, pregnancy, and others, contributed to the development of aHUS in 69% of cases (14). In our case, a high-dose corticosteroid did not improve the serum complement levels, suggesting that the low serum complement levels unlikely reflected the SLE activity. Because our patient had undergone repeated hospitalization for pneumonia since her teenage years, she might have had congenital immune disorders before the onset of SLE. An analysis of the abnormalities of complement regulatory factors and/or genes is necessary to confirm the de-
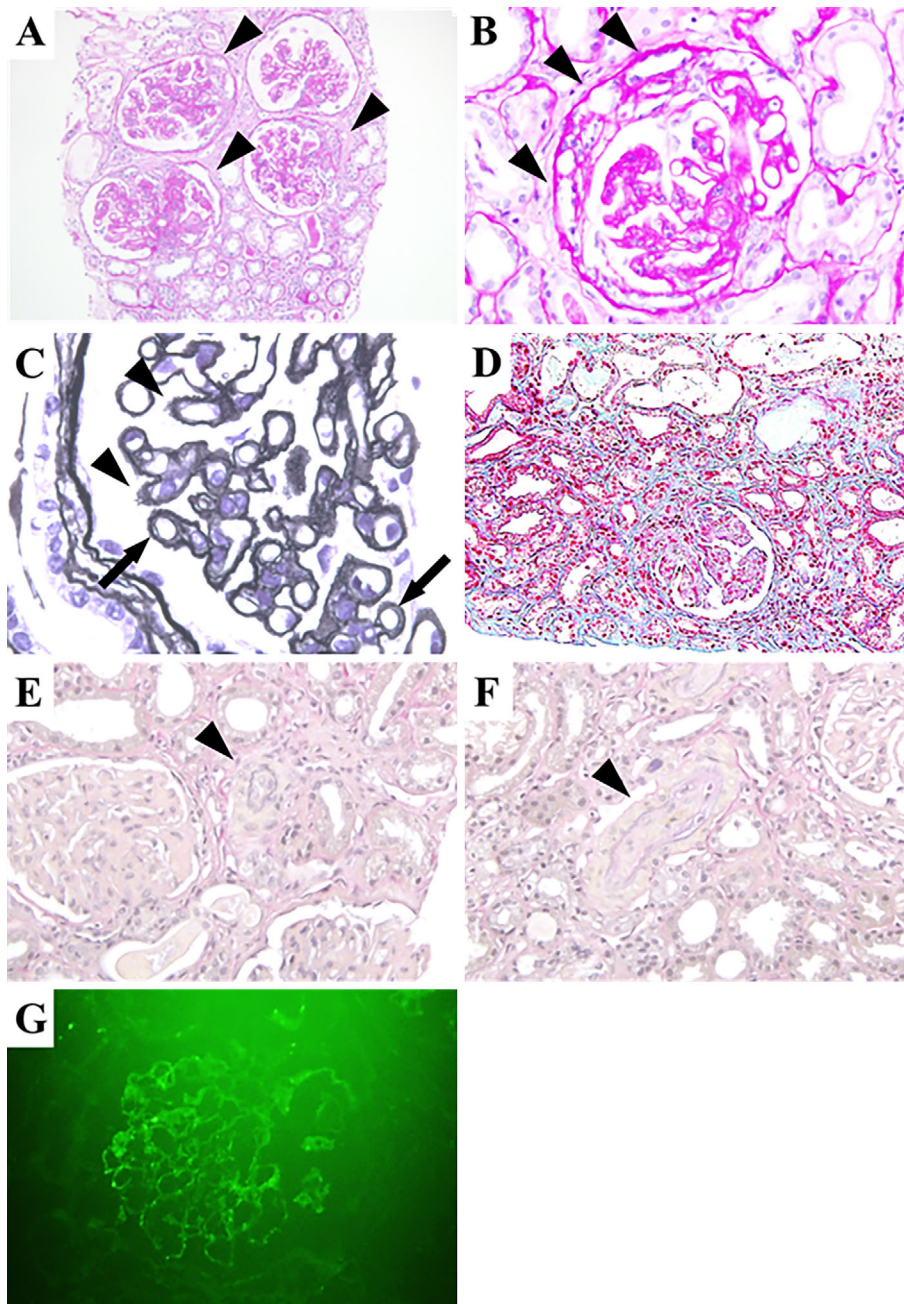
Figure 2.   Light microscopic and immunofluorescent findings of the kidney biopsy. Periodic acid-Schiff (PAS) staining (A, B) shows that most of the glomeruli tend to collapse and exhibit wrinkling changes in the capillary walls and luminal narrowing associated with organized crescents (pseudotubulization) (arrowheads). Periodic acid-methenamine-silver (PAM) staining (C) shows double contouring (arrows) as well as thickening, spike formation, and a bubbly appearance (arrowheads) in the capillary walls. Masson's trichrome staining (D) shows diffuse interstitial fibrosis and tubular atrophy in approximately 50% of the tubulointerstitial areas. Elastica van Gieson (EVG) staining (E, F) shows swelling of the endothelium along with luminal narrowing and obstruction (arrowhead) in the arteriole (E) and small artery (F). Immunofluorescence shows slightly positive staining for IgG in the capillaries and part of the mesangial region of the glomerulus. Original magnification, ×200 (A, E, F, G), ×400 (B, D), and 1,000 (C), respectively.

fects in the complement pathway.

Eculizumab is a humanized monoclonal antibody against C5 that inhibits the cleavage of C5 into C5a and C5b and suppresses the activation of a late step of the complement cascade (15). Eculizumab was recently reported to be effec-

tive for treating TMA caused by SLE (16) as well as complement-mediated disorders (17-19). While the official indication of eculizumab is complement-mediated HUS in Japan, we suspect that aHUS was involved in the etiology of TMA in this patient. We therefore decided to start eculizu-
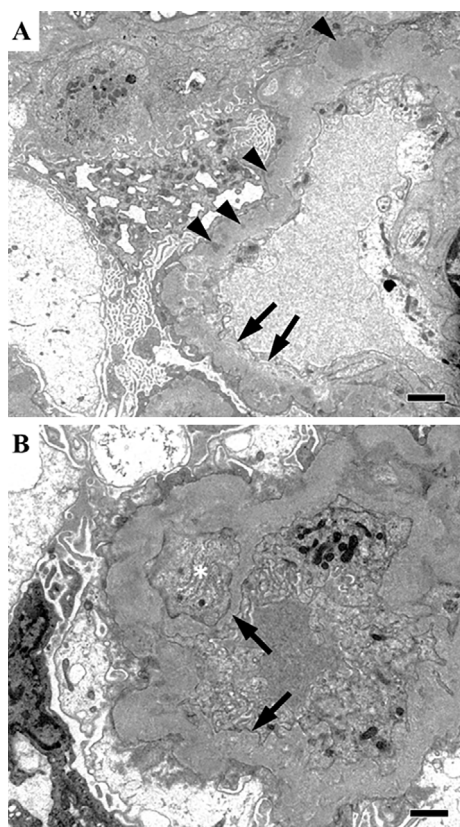
Figure 3. **Electron microscopic findings of the renal biopsy. Electron microscopy reveals thickening of the glomerular basement membrane (A, B), partial effacement of the foot process (A), slight enlargement of the subendothelial space (A, arrows), swelling of the endothelial cells (B), mesangial interposition (B, ∗), and a newly synthesized basement membrane (B, arrows). Electron-dense deposits of various sizes are seen in the subepithelial and intramembranous regions (A, arrowheads) but not in the subendothelial regions. Bar: 5 μm (A) and 1 μm (B).**

mab, which improved the clinical condition in our patient, although hemodialysis could not be discontinued. Zuber et al. recommended that eculizumab be considered in cases of aHUS where the normalization of platelets and LDH or a >25% improvement in the serum creatinine value are not found despite treatment with plasma exchange for 5 consecutive days or in cases of recurrence (20). Legendre et al. reported administering eculizumab to 20 cases of aHUS that required plasma exchange for 26 weeks, with 16 cases (80%) showing no recurrence for at least 62 weeks (19). Furthermore, it has been reported that earlier treatment with eculizumab led to more effective improvement in the renal function, suggesting that the early introduction of eculizumab was desirable for maintaining the renal function (19, 21). Accordingly, it is recommended that eculizumab be started without waiting for test results regarding abnormalities of complement regulatory factors when aHUS is suspected clinically (1).

In summary, we experienced a case of class V lupus nephritis associated with TMA possibly caused by aHUS in which a rare condition of diffuse pseudotubulization after the organization of crescent formation due to TMA was found after the successful treatment of TMA activity with eculizumab.

The authors state that they have no Conflict of Interest (COI).

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