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【 CASE REPORT 】

Exposure of Thomsen-Friedenreich Antigen on the Renal Tubules of a Patient with *Capnocytophaga* Infection-induced Acute Kidney Injury

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Abstract:
Infections with neuraminidase-producing bacteria can lead to acute kidney injury (AKI). We herein report a 74-year-old woman who developed AKI in the course of *Capnocytophaga* infection, a neuraminidase-producing bacterium. A renal biopsy showed tubulointerstitial injury accompanied by specific binding of fluorescence-conjugated peanut lectin to the tubular epithelial cells, suggesting exposure of Thomsen-Friedenreich antigen (T-antigen) on the tubules. Although AKI is often observed in patients infected with *Capnocytophaga*, little is known about its etiology and associated pathology. This case suggests that tubulointerstitial injury caused by neuraminidase production and resultant T-antigen exposure is a mechanism of *Capnocytophaga* infection-induced AKI.

Key words: acute kidney injury, *Capnocytophaga*, Thomsen-Friedenreich antigen, thrombotic microangiopathy

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Introduction

Capnocytophaga sp. is a Gram-negative rod-shaped bacterium found in the normal oral flora of dogs and cats and is often isolated from patients who develop infections after being bitten or scratched by such animals (1). Infections caused by this bacterium often present as life-threatening sepsis with hypotension (septic shock) and/or septic disseminated intravascular coagulation (DIC), and the mortality rate of such patients is reportedly high (2).

Acute kidney injury (AKI) is also often seen in the course of *Capnocytophaga* infection and sometimes necessitates renal replacement therapy. Hypotension and DIC are considered prominent causes of *Capnocytophaga* infection-induced AKI (2), but the occurrence of thrombotic microangiopathy (TMA) or acute tubular necrosis has been described in some reports (3, 4). However, little is known about the mechanisms or pathology associated with *Capnocytophaga* infection-induced AKI.

There are various causes of TMA, including bacterial infections. Shiga toxin-producing *Escherichia coli* is the most well-known cause of TMA with diarrhea. Infected patients often develop AKI, and such patients are diagnosed with hemolytic uremic syndrome. In contrast, infections with neuraminidase-producing bacteria can also cause TMA (and other forms of renal injury) without diarrhea, in which exposure of Thomsen-Friedenreich antigen (T-antigen) is considered to play an important role (5).

We herein report a patient with AKI caused by an infection with *Capnocytophaga*, which is a neuraminidase-producing bacterium. This patient presented with septic shock and DIC; however, a renal biopsy showed tubulointerstitial injury with specific binding of fluorescein isothiocyanate (FITC)-conjugated peanut lectin to the tubular epithelium, suggesting that T-antigen exposure was involved in the course of AKI.

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Table. **Laboratory Data of the Patient.**

Arterial blood gas analysis			**Biochemistry**		
pH	7.37		Blood urea nitrogen	45.5	mg/dL
HCO_3-	16.4	mEq/L	Creatinine	3.34	mg/dL
Base excess	-7.5	mmol/L	Total protein	6.5	g/dL
Anion gap	24.6	mEq/L	Albumin	3.0	g/dL
			Aspartate aminotransferase	163	U/L
Complete blood count			Alanine aminotransferase	59	U/L
WBC	6,480	/μL	Lactate dehydrogenase	1,167	U/L
Hemoglobin	14.8	g/dL	Total bilirubin	3.9	mg/dL
Platelet	13,000	/μL	Creatine kinase	659	U/L
			Sodium	138	mEq/L
Serology			Potassium	4.5	mEq/L
IgG	976	mg/dL	Chloride	97	mEq/L
IgA	231	mg/dL	Calcium	7.0	mg/dL
IgM	41	mg/dL	Phosphate	6.4	mg/dL
Complement C3	96	mg/dL	Glycosylated hemoglobin	6.1	%
Complement C4	22.1	mg/dL	CRP	29.3	mg/dL
ANA titer	1:80				
Procalcitonin	64.6	ng/mL	**Coagulation**		
Ferritin	1,051.1	ng/mL	APTT	109.5	s
ADAMTS13 activity*	123	%	PT-INR	3.07	
PA-IgG*	22	ng/10^7 cells	FDP	261.2	μg/mL
Direct Coombs test*	Positive		Antithrombin III	43	%

*Data were obtained about three years after the onset of *Capnocytophaga* infection.

ADAMTS13: a disintegrin-like and metalloproteinase with thrombospondin type 1 motifs 13, ANA: antinuclear antibody, APTT: activated partial thromboplastin time, CRP: C-reactive protein, FDP: fibrinogen degradation products, Ig: immunoglobulin, PA: platelet-associated, PT-INR: prothrombin time-international normalized ratio, WBC: white blood cell

Case Report

A 74-year-old woman who was being treated for diabetes mellitus presented with a fever, nausea, and diarrhea, and was transferred to our hospital. Her baseline renal function was normal, i.e. proteinuria was not noted, and her serum creatinine level was 0.54 mg/dL. On admission, her blood pressure was 76/40 mmHg, and her body temperature was 34.5 °C. She had been bitten on her finger by her dog three days before admission.

Her laboratory test results are summarized in Table. A blood analysis showed a hemolytic reaction. There was prominent thrombocytopenia (platelet count: 1.3×10^4/μL) accompanied by abnormalities of the blood coagulation system, suggesting DIC. She also had severe AKI (serum creatinine: 3.34 mg/dL; blood urea nitrogen: 45.5 mg/dL) and liver injury. A substantial increase in C-reactive protein and procalcitonin were noted, whereas the levels of immunoglobulins (Igs) and complements were normal. Her antinuclear antibody titer was only slightly increased. A urinalysis could not be performed because she was anuric. A stool culture only detected normal flora.

The patient's clinical course is shown in Fig. 1. She was diagnosed with septic shock and DIC and immediately began antibiotics therapy with meropenem and clindamycin, as well as the administration of vasopressors, thrombomodulin alfa, and platelet transfusion. However, she developed purpura fulminans in her face and fingers and showed further progression of AKI. Renal replacement therapy (initially continuous hemodiafiltration followed by intermittent hemodialysis) was thus performed from day 2 of hospitalization. Several days later, *Capnocytophaga* sp. was isolated from her blood culture, and the antibiotics were changed to ampicillin/sulbactam.

Her general condition subsequently improved, and renal replacement therapy was ended 12 days after initiation; however, her renal dysfunction (serum creatinine, 1.1 mg/dL; estimated glomerular filtration rate, 37.9 mL/min/1.73 m^2) and massive proteinuria (2-3 grams per day) continued. Although schistocytes were not detected in a blood smear, hemolytic reactions were repeatedly observed on a routine blood examination. A urinalysis showed only minor isomorphic hematuria (1-4 red blood cells/high-power field), but urinary occult blood was positive, suggesting the possibility of a false-positive result.

A light microscopy analysis of sections from a renal biopsy performed percutaneously about 2 months after disease onset showed 15 glomeruli without signs of diabetic nephropathy. Four glomeruli were globally sclerotic, and one of the remaining glomeruli was segmentally sclerotic. In addition, substantial tubulointerstitial injury was observed (Fig. 2A), and the deposition of hemosiderin was observed in some tubular epithelial cells (Fig. 2B). The nonsclerotic glomeruli

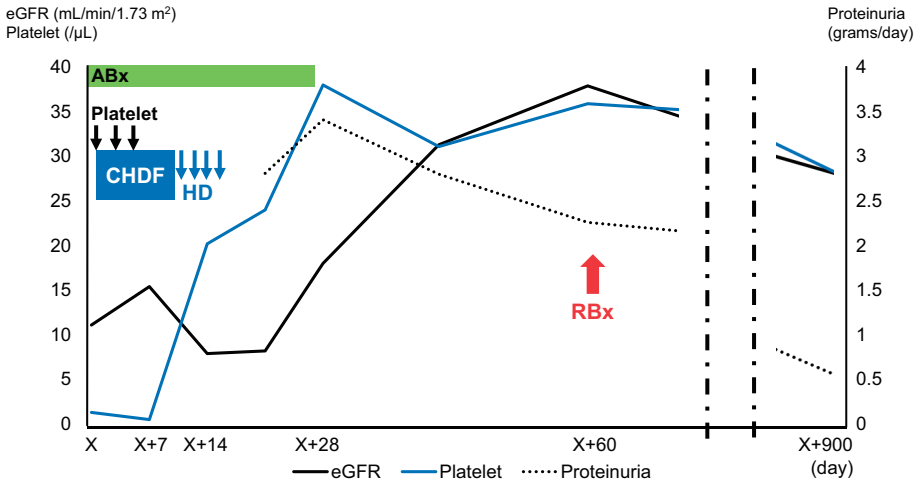

Figure 1. **The patient's clinical course. Antibiotics therapy and platelet transfusion were started immediately after hospitalization (day X). Owing to the progression of her acute kidney injury, renal replacement therapy was started from day 2. Because renal dysfunction and proteinuria continued after the cessation of renal replacement therapy, a renal biopsy was performed about two months after the disease onset. ABx: antibiotics, CHDF: continuous hemodiafiltration, eGFR: estimated glomerular filtration rate, HD: hemodialysis, RBx: renal biopsy**

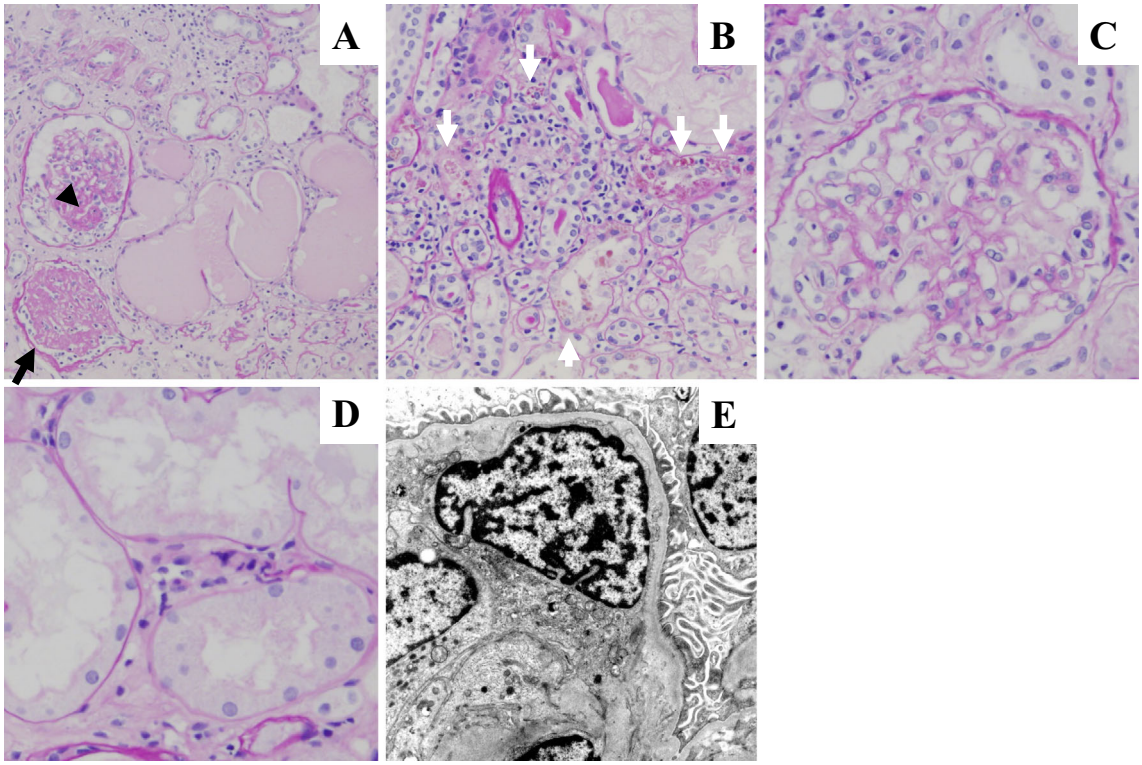

Figure 2. **Histological features of the patient's renal biopsy. (A) Mononuclear cell infiltration, edema of the interstitium, and dilation of the tubules are shown. One glomerulus is obsolescent (arrow), and another is segmentally sclerotic (arrowhead). (B) The deposition of hemosiderin (white arrows) is seen in some tubular epithelial cells. (A, B) Periodic acid-Schiff staining. (C) No proliferative changes or thickening of the glomerular capillary walls was observed in a nonsclerotic glomerulus. (D) Neither capillaritis nor thrombi were observed in the peritubular capillaries (original magnification, A: 100×; B-C: 200×; D: 400×). (E) An electron microscopic image showing the absence of electron-dense deposits and signs of thrombotic microangiopathy, such as glomerular endothelial swelling, widening of the subendothelial space, and double contours of the glomerular basement membrane.**

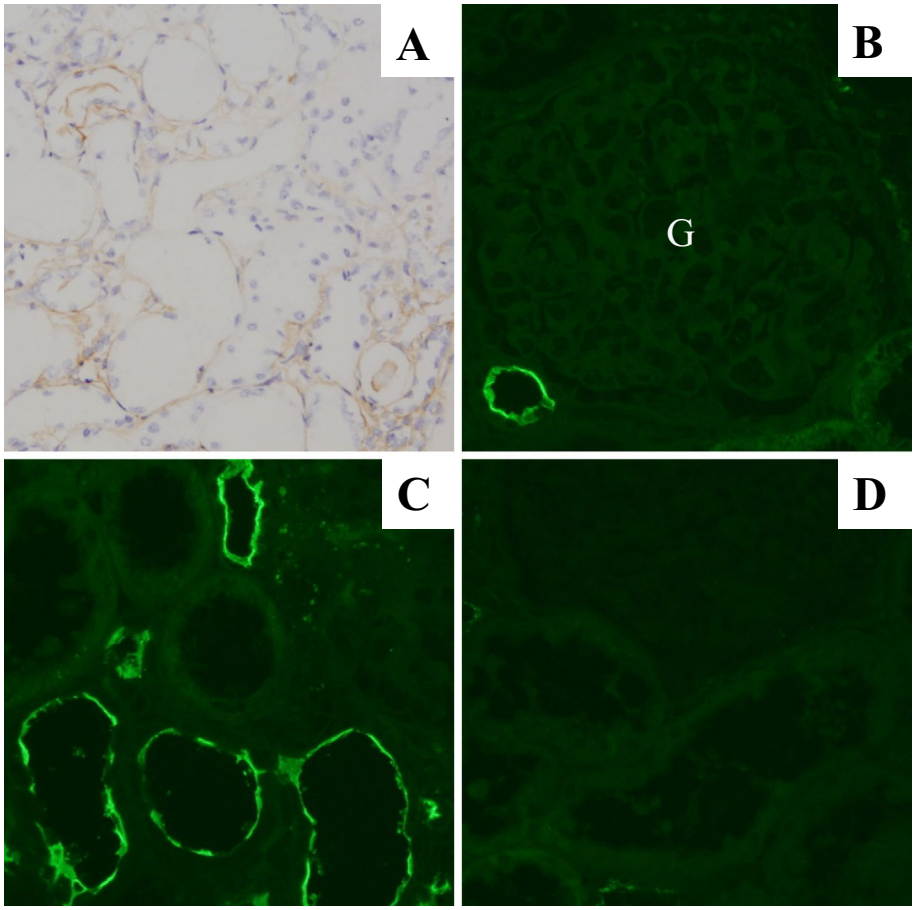

Figure 3. **Exposure of Thomsen-Friedenreich antigen on tubular epithelial cells. (A) Immunoglobulin M deposition on tubular epithelial cells shown by immonoperoxidase staining. (B-C) Staining with fluorescein isothiocyanate-conjugated peanut lectin (Vector Laboratories, Burlingame, USA; green). Although no specific binding of peanut lectin to a glomerulus was observed (B), some tubular epithelial cells show specific binding to peanut lectin, suggesting Thomsen-Friedenreich antigen on these cells (C). (D) There was no specific binding of peanut lectin to tubular epithelial cells of the normal control renal tissue (original magnification, A-D: 200×). G: glomerulus**

did not show proliferative changes or thickening of the glomerular capillary walls (Fig. 2C). Neither capillaritis nor thrombi were observed in the peritubular capillaries (Fig. 2D). Immunostaining demonstrated no Ig or complement deposition in the glomeruli, and electron microscopy did not show any electron-dense deposits or signs of TMA, such as glomerular endothelial swelling, widening of the subendothelial space, or double contours of the glomerular basement membrane (Fig. 2E). In contrast, focal IgM deposition was observed on tubular epithelial cells (Fig. 3A). Furthermore, FITC-conjugated peanut lectin was found to specifically bind to the tubular epithelial cells (Fig. 3B, C), whereas there was no specific binding of FITC-conjugated peanut lectin on tubular epithelial cells of the normal control renal tissue (Fig. 3D).

Given the above findings, a pathological diagnosis of acute tubular injury with T-antigen exposure was made. The patient was thereafter treated conservatively with an angiotensin II receptor blocker. Two years after the renal biopsy, her serum creatinine level was 1.32 mg/dL (estimated glomerular filtration rate, 30.4 mL/min/1.73 m^2), and her protein excretion rate was 0.5 g/day, with no sediment abnormalities on a urine examination.

Discussion

Patients with *Capnocytophaga* infection often develop AKI, but the associated mechanism and pathology have not been well investigated. There has been a previous case of AKI caused by *Capnocytophaga* bacteremia in which mesangiolysis as well as tubular necrosis were observed (4). The present case was complicated with septic shock and DIC, both of which might have affected the renal pathology; however, to our knowledge, this is the first case of a patient showing tubulointerstitial injury accompanied by exposure of T-antigen on the renal tissue.

Patients infected with neuraminidase-producing bacteria can develop TMA. It is considered in this setting that the T-antigen, which is hidden under normal conditions by neuraminic acid, is exposed by neuraminidase activity. Preformed IgM antibodies of the infected host then bind to the exposed T-antigen on the surface of red blood cells, plate-

lets, and endothelial cells, thereby initiating a cascade of events leading to TMA (5). *Streptococcus pneumoniae* infection is a well-known cause of such TMA, which is reportedly often associated with sepsis/bacteremia (6). However, there are several pathogens that have neuraminidase activity, and interestingly, *Capnocytophaga* sp. is one such type of bacteria (7, 8).

S. pyogenes is another neuraminidase-producing bacterium that may cause TMA via similar mechanisms (9). Indeed, we recently encountered an interesting case of a patient with *S. pyogenes* infection, in which concurrence of TMA with positive tubular FITC-conjugated peanut lectin staining and poststreptococcal acute glomerulonephritis with positive glomerular staining of nephritis-associated plasmin receptor, a nephritogenic streptococcal protein, and related plasmin activity (10) was observed (manuscript in preparation). T-antigen exposure-induced TMA can thus occur together with other forms of renal injury.

Peanut lectin has been shown to have high affinity for T-antigen, so exposure of T-antigens can be assessed using labeled peanut lectin as a probe. Using biotin-labeled peanut lectin and FITC-conjugated streptavidin as probes, Shimizu et al. demonstrated the exposure of T-antigen in glomeruli as well as the tubules of a patient with *S. pyogenes*-associated hemolytic uremic syndrome (9). In contrast, using FITC-labeled peanut lectin as a probe, Alon et al. demonstrated the presence of T-antigen exposure only in the tubular epithelium of a patient with *S. pneumoniae*-associated hemolytic uremic syndrome, similar to the findings in the present patient (11). Our patient did not show typical pathological findings of TMA; however, an increase in the lactate dehydrogenase level, together with repeated hemolytic reactions on a blood examination, and a possible false-positive result of urinary occult blood, all of which were suggestive of TMA, were observed. In addition, the deposition of hemosiderin in tubular epithelial cells, which is a possible sign of hemolysis, was also observed. Schistocytes were not detected in the patient's blood smear, but this may be due to the low sensitivity of this test. Thus, it is possible that TMA lesions were actually present but were not detected by the percutaneous needle biopsy. It is also possible that the timing at which the renal biopsy was performed affected the renal histology, as the biopsy of the present patient was not performed in the early clinical course.

Another important issue is regarding treatment options. Although we initially diagnosed the patient with septic shock and DIC, the possibility of thrombotic thrombocytopenic purpura could not be completely ruled out. However, as secondary TMA induced by infection was more likely, we did not perform plasmapheresis. Instead, we considered platelet transfusion necessary because the patient showed severe thrombocytopenia ($1.3\times10^4/\mu L$) on admission, whereas we had to perform invasive procedures, such as central venous catheter insertion. When treating patients with *Capnocytophaga* infection, it may be more favorable to choose washed blood products for transfusion, as the transfusion of blood products containing plasma may be harmful due to the presence of donor IgM antibodies against the T-antigen. Further studies of such cases to investigate safer and more effective treatments will be required in the future.

Several limitations associated with the present study warrant mention. We did not obtain key laboratory data in the acute disease phase, such as a disintegrin-like and metalloproteinase with thrombospondin type 1 motifs 13 (ADAMTS13) activity, platelet-associated IgG level, haptoglobin level, and the Coombs test results. It should be also stressed that we were unable to establish a definitive diagnosis of TMA due to the lack of these data. However, we measured these factors about three years after the disease onset, and there was no decrease in the ADAMTS13 activity, and the level of platelet-associated IgG was within the reference range. While the Coombs test was positive, the patient was not anemic at that time. The Coombs test is often positive in patients with *S. pneumoniae* infection-induced TMA (9). We therefore considered that the Coombs test might have become positive in association with *Capnocytophaga* infection, and the patient's Coombs test has continuously been positive ever since. Hemolysis is known to occur in association with DIC, and there is a possibility that our patient had TMA with DIC, and her renal damage may have simply been caused by sepsis-induced ATN.

In conclusion, we encountered a patient who developed AKI during the course of *Capnocytophaga* infection, in which a renal biopsy showed tubulointerstitial injury accompanied by T-antigen exposure. This case suggests that tubulointerstitial injury caused by neuraminidase production and resultant T-antigen exposure is a mechanism of *Capnocytophaga* infection-induced AKI.

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

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