Atypical reduction of plasma ADAMTS13 activity by a non-IgG-type inhibitor in a patient with hemolytic uremic syndrome caused by Shiga toxin-producing *Escherichia coli*

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Abstract

Thrombotic microangiopathies include hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP). Measurement of plasma levels of “a disintegrin-like and metalloproteinase with thrombospondin type 1 motifs 13” (ADAMTS13) activity can distinguish HUS from TTP. Reduced plasma ADAMTS13 activity (< 10% normal range) is atypical for HUS, but not for TTP. However, we detected reduced ADAMTS13 activity in a patient with Shiga toxin-producing *Escherichia coli*-associated HUS caused by non-IgG anti-ADMTS13 autoantibodies. Furthermore, the patient exhibited possible genetic abnormalities associated with atypical HUS. The patient fully recovered after administration of supportive therapy. To the best of our knowledge, very few cases of STEC-HUS with reduced ADAMTS13 activity have been reported; thus far, none have described the presence of non-IgG anti-ADMTS13 autoantibodies. Therefore, we suggest that anti-ADAMTS13 analyses should be performed in patients diagnosed with STEC-HUS, especially in those who present with prolonged healing or unexpected clinical symptoms.

Keywords A disintegrin-like and metalloproteinase with thrombospondin type 1 motifs 13 · Atypical hemolytic uremic syndrome · Shiga toxin-producing *Escherichia coli*-associated hemolytic uremic syndrome · Thrombotic microangiopathy · Thrombotic thrombocytopenic purpura

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Introduction

It is difficult to distinguish hemolytic uremic syndrome (HUS) from thrombotic thrombocytopenic purpura (TTP) because of their clinical similarities [1]. Acute renal injury, microangiopathic hemolytic anemia, and thrombocytopenia characterize HUS [2]. In addition to these three symptoms, neurological symptoms are present in patients with TTP, but are undetectable in 35% of affected patients; thus, it is difficult to distinguish TTP and HUS solely on the basis of a patient's symptoms [3]. However, this distinction can be made by measurement of ADAMTS13 activity [4]: ADAMTS13 activity is generally reduced in patients with TTP, compared with that in patients with HUS. HUS has been observed in patients with lower ADAMTS13 activities, although the values are not significantly different from the normal range [4]. If ADAMTS13 activity is reduced to < 10% of the normal range (considered a significant reduction), the patient is diagnosed with TTP [4]. Furthermore,

if an ADAMTS13 inhibitor is detected, the patient is diagnosed with acquired TTP [4].

Shiga toxin-producing *Escherichia coli* (STEC)-HUS occurs after hemorrhagic enteritis caused by enterohemorrhagic *E. coli* [5]. In STEC-HUS, Shiga toxin (Stx) is released in the intestine and circulates in the bloodstream [6]. In vitro, Stx interacts with the surfaces of human endothelial cells and leukocytes, which suggests that a similar mechanism is present in the bloodstream. Vascular damage induced by Stx promotes cytokine and chemokine secretion by endothelial cells [7]. The primary target organ is the kidney, in which various cells express high levels of surface receptors targeted by Stx; these cells are, therefore, susceptible to toxin-mediated injury. [7]. Notably, Stx directly impairs the endothelium and changes endothelial function through production of inflammatory cytokines, chemokines, and adhesion factors, as well as by induction of hypercoagulability [7]. Thus, reduction of ADAMTS13 activity, caused by anti-ADAMTS13 autoantibodies, is not detected in patients with STEC-HUS [6].

Antibiotics are not recommended in the United States for treatment of patients with STEC-HUS with infectious gastroenteritis [8]. Therefore, we do not use antibiotics for treatment of such patients in our hospital, as this may cause the release of toxins and lytic phages [9]. In these patients, appropriate early volume expansion with intravenous fluid, including sodium, may avoid oliguria, anuria, and the requirement for dialysis [10].

Here, we describe treatment of a patient with STEC-HUS, in whom ADAMTS13 activity was reduced by non-IgG anti-ADAMTS13 autoantibodies. In addition, the patient exhibited possible genetic abnormalities caused by a rare mutation of membrane cofactor protein (MCP), which was detected in analysis of atypical HUS (aHUS).

Case report

A 21-year-old woman exhibited fever, lower abdominal pain, and diarrhea after ingesting raw meat. She was prescribed an antibiotic and an antifatulent at another clinic. She reported bloody diarrhea ten times daily and experienced increasing fatigue. Laboratory analysis revealed severe anemia [hemoglobin (Hgb), 6.0 g/dL], reduced platelet (Plt) count ($3.1 \times 10^4/\mu\text{L}$), and renal injury (serum creatinine, 6.17 mg/dL). She was admitted to our hospital 10 days after the onset of symptoms. She reported no notable medical history, no family history of disease, no medications, and no allergic disorders.

She lost 1.0 kg of body weight and exhibited palpebral conjunctiva, as well as mild jaundice without edema. She maintained consciousness and had no fever or abdominal pain; bloody diarrhea resolved upon hospitalization. Her

laboratory findings were as follows: Hgb, 4.9 g/dL; Plt count, $1.9 \times 10^4/\mu\text{L}$; lactate dehydrogenase, 3302 U/L; haptoglobin, < 10 mg/dL; negative direct Coombs test; and liver and renal injury (Table 1). A peripheral blood smear showed numerous crushed erythrocytes. The patient exhibited the essential diagnostic criteria of microangiopathic hemolytic anemia and thrombocytopenia (TMA) with severe renal failure without neurological abnormalities. Although neurologic abnormalities are commonly considered to be characteristic of TTP and renal dysfunction is considered to be characteristic of HUS, we regarded the patient's symptoms as indicative of a typical clinical course of HUS.

The patient's stool was cultured after she had been prescribed an antibiotic. Thus, enterohemorrhagic *E. coli* was not detected. However, the patient had two positive results for anti-O157 *E. coli* lipopolysaccharide antibody. Therefore, we diagnosed her with STEC-HUS. The patient was hospitalized and infusion therapy was implemented; erythrocytes were transfused to treat severe anemia. Antibiotics were not used, and the patient underwent volume expansion with intravenous fluid; she was instructed to fast. These treatments are generally regarded as conventional supportive therapy [10].

We next measured the level of plasma ADAMTS13 activity in the patient, as well as the level of an ADAMTS13 inhibitor at admission. Using an ADAMTS13-act-ELISA kit (Technoclone, Vienna, Austria). This kit was able to detect the activity of whole ADAMTS13 inhibitor molecules. The plasma level of ADAMTS13 activity was 9.3% of normal, whereas that of the ADAMTS13 inhibitor was 4.2 Bethesda Units/mL. These data led us to a diagnosis of acquired TTP, with STEC infection as the underlying disease. Therefore, the patient's final diagnosis was STEC-HUS, although we considered the possibility of acquired TTP. We noted that STEC-HUS and acquired TTP were present in the patient, although these appeared to be inconsistent diagnoses; thus, we confirmed the ADAMTS13 inhibitor properties. Anti-ADAMTS13 IgG antibodies were measured using the Technozym ADAMTS-13 INH kit (Technoclone). However, we did not detect IgG antibodies against ADAMTS13. Therefore, the ADAMTS13 inhibitor that we initially detected was judged to be a non-IgG antibody against ADAMTS13.

Conventional supportive treatment (fluid therapy and RBC transfusion) improved the patient's kidney function, and she was discharged after 13 days (Fig. 1). Immediately before discharge, her ADAMTS13 activity had increased to > 100%, and the titer of ADAMTS13 inhibitor had decreased below the limit of detection. Moreover, the patient lacked detectable symptoms, and her Plt count and renal function had recovered to normal.

After discharge, she underwent screening to determine whether HUS had been induced by an infectious disease. Plasma complement factor H (CFH) autoantibody

Table 1 Laboratory values

(Urine)			
Density	1.014		1.005–1.030
pH	5.0	↓	6.0–6.5
Protein	3+	↑	Negative
Blood	3+	↑	Negative
Red blood cell (/HPF)	10–19	↑	0–4
Hyaline cast (/HPF)	10–19	↑	Negative
Granular cast (/LPF)	0–1	↑	Negative
Epithelial cast (/LPF)	1–4	↑	Negative
Waxy cast (/LPF)	1–4	↑	Negative
Pro/Cre ratio (g/g gCr)	3.79	↑	<0.15
NAG (U/L)	44.6	↑	1.0–6.3
β2MG (mg/L)	0.31		0.9–1.9
Gestation test	Negative		Negative
(Blood)			
Leukocyte count (/μg)	8830		3040–8540
Erythrocyte count (×10 ³ /μL)	1720	↓	3780–4990
Hemoglobin (g/dL)	4.9	↓	10.8–14.9
Hematocrit (%)	14.0	↓	35.6–45.4
MCV (fL)	90.9		85.0–101.0
MCHC (g/dL)	33.1		30.7–34.0
Platelet count (×10 ³ /μL)	19	↓	150–360
Neutrophil (%)	64.0		38.3–71.1
Lymphocyte (%)	23.5	↓	25.0–45.0
Monocyte (%)	11.6	↑	4.0–7.0
PT-INR	1.03		
APTT (s)	32.1		26.9–38.1
Fibrinogen (mg/dL)	195.7	↓	200.0–400.0
FDP (μg/mL)	21.1	↑	≤5.0
D-Dimer (μg/mL)	6.2	↑	≤1.0
Direct Coombs	Negative		Negative
Ferritin (ng/ml)	1716.4	↑	11.0–128.9
TSAT (%)	41.7	↑	35
Haptoglobin (mg/dL)	<10	↓	19–170
Total bilirubin (mg/dL)	2.3	↑	0.3–1.2
Direct bilirubin (mg/dL)	0.4	↑	0.1–0.3
AST (U/L)	128	↑	13–33
ALT (U/L)	70	↑	8–42
LDH (U/L)	3202	↑	119–229
Alkaline phosphatase (U/L)	138		115–359
γ-GTP (U/L)	10		9–32
Amylase (U/L)	76		44–132
Creatine kinase (U/L)	159		45–163
Sodium (mEq/L)	130	↓	138–146
Potassium (mEq/L)	3.3	↓	3.6–4.9
Chloride (mEq/L)	99		99–109
Corrected serum calcium (mg/dL)	9.2		8.6–10.4
Phosphate (mg/dL)	3.7		2.5–4.7
Total protein (g/dL)	5.4	↓	6.7–8.3
Albumin (g/dL)	2.6	↓	4.0–5.0
Uric acid (mg/dL)	12.8	↑	2.3–7.0

Table 1 (continued)

Urea nitrogen (mg/dL)	92.7	↑	8.0–22.0
Creatine (mg/dL)	6.17	↑	0.40–0.70
eGFR (mL/min/1.73 m ²)	8.2	↓	≥90
CRP (mg/dL)	1.11	↑	≤0.20
Procalcitonin (ng/mL)	0.67	↑	≤0.49
IgG (mg/dL)	929		870–1700
IgA (mg/dL)	210		110–410
IgM (mg/dL)	142		46–260
CH50 (U/mL)	39.0		30.0–46.0
C3 (mg/dL)	100		86–160
C4 (mg/dL)	19		17–45
PR3-ANCA (U/mL)	<3.5		<3.5
MPO-ANCA (U/mL)	<3.5		<3.5
Anti-nuclear antibody	<×80		<×80
Other antibodies	Negative		Negative
Hepatitis B surface antigen	Negative		Negative
Hepatitis B virus antibody	Negative		Negative
Hepatitis B core antigen	Negative		Negative
Hepatitis C antibody	Negative		Negative
HIV antibody	Negative		Negative
CMV (CF)	Negative		Negative
HSV	Negative		Negative
Epstein–Barr virus	Negative		Negative
Parvo virus	Negative		Negative
ADAMTS13 activity (%)	9.3	↓	50–150
Anti-ADAMTS13 inhibitor (BU/mL)	4.2	↑	<0.5
Anti-ADAMTS13 IgG	Negative		Negative

HPF high-power field, *LPF* low-power field, *Pro/Cre Ratio* protein/creatinine ratio, *NAG* *N*-acetyl-β-D-glucosaminidase, *β2MG* β2-microglobulin, *FDP* fibrinogen degradation product, *MCV* mean corpuscular volume, *MCHC* mean corpuscular hemoglobin, *PT-INR* international normalized ratio of prothrombin time, *APTT* activated partial thromboplastin time, *FDP* fibrin degradation products, *TSAT* transferrin saturation, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *LDH* lactate dehydrogenase, *γ-GTP* γ-glutamyl transpeptidase, *eGFR* estimated glomerular filtration rate, *IgG* immunoglobulin G, *IgA* immunoglobulin A, *IgM* immunoglobulin M, *PR3-ANCA* proteinase 3-anti-neutrophil cytoplasmic antibody, *MPO-ANCA*, myeloperoxidase-anti-neutrophil cytoplasmic antibody, *HIV* human immunodeficiency virus, *CFH* complement factor H

was not detected using a CFH IgG ELISA Kit (KA1477; Abnova, Taipei City, Taiwan). We performed whole exome sequencing of genes associated with aHUS as follows: *CFH*; membrane cofactor protein (*MCP*); complement factor I (*CFI*), *C3*; complement factor B (*CFB*); diacylglycerol kinase epsilon (*DGKE*); and thrombomodulin (*THBD*). These analyses detected a rare mutation, p.Ala311Val (c.932C>T), in exon 8 of *MCP*; this mutation had been previously detected. [11, 12].

Fig. 1 Patient's clinical course

Discussion

Here, we described a patient with STEC-HUS who exhibited reduced ADAMTS13 activity and an increased titer of non-IgG anti-ADAMTS13 autoantibodies during hospitalization. Furthermore, the patient harbored a rare p.Ala311Val (c.932C>T) mutation in *MCP*. To the best of our knowledge, there have been no other reports of patients with these particular comorbidities.

The patient's coagulation markers increased upon hospitalization (Table 1). Disseminated intravascular coagulation (DIC) and TMA can be distinguished as follows: DIC exhibits extreme abnormalities in clotting markers, whereas TMA exhibits only mild abnormalities in these markers [13]. However, DIC can be evoked by TMA, such as STEC-HUS [13]. Therefore, it is often difficult to distinguish DIC during early TMA.

Most patients with acquired TTP with severe ADAMTS13 deficiency have circulating anti-ADAMTS13 IgG autoantibodies that can neutralize ADAMTS13 activity [14]. Certain infectious diseases, including STEC, cause an autoimmune reaction to ADAMTS13 [15]. When acquired TTP occurs as a result of infection, cross-reactivity of CD4-positive T cells is suspected, mediated by a protein derived from a microorganism that produces a protein homologous to ADAMTS13 [16]. Moreover, some TTP patients with severe ADAMTS13 deficiency lack ADAMTS13-neutralizing autoantibodies [17]. In general, in patients with acquired TTP, ADAMTS13-neutralizing autoantibodies reduce the activity of ADAMTS13 [16]. However, in the present case, the patient's autoantibodies were not IgGs, but may have been IgA or IgM, which may explain the inhibition of ADAMTS13 activity.

Unfortunately, we were limited by the volumes of blood collected from the patient at the early and severe clinical stages, and could not conduct further analyses to precisely identify the antibody.

The patient's pathophysiological condition did not strictly meet the accepted definition of acquired TTP. When ADAMTS13 activity is undetectable, abnormally large von Willebrand factor multimers in plasma can exhibit greater reactivity with Plt and cause disseminated platelet thrombi that are characteristic of TTP [18]. Alternatively, proteases may be activated due to STEC infection; in the present case, this may have led to the remarkable reduction (< 10%) of ADAMTS13 activity, relative to that detected in patients with TTP. This may explain the DIC-like disease course, which resulted from the reduction in ADAMTS13 activity. However, we were unable to conduct further analyses of these putative inhibitors because of the limited blood sample, as indicated in the prior paragraph. Contrary to expectations, the patient's ADAMTS13 activity had decreased to the level present in patients with acquired TTP; therefore, we re-measured ADAMTS13 with the same sample. The findings showed good reproducibility of the patient's ADAMTS13 reduced activity. Next, we assessed whether the ADAMTS13 inhibitor in that sample was IgG, and found that it was not. Because of these repeated examinations at early and severe clinical phases, the samples were insufficient for further analyses, as noted in a prior paragraph.

The results of aHUS analysis revealed a rare mutation, p.Ala311Val, in exon 8 of *MCP*. Although this mutation has been found in patients with aHUS, its pathological significance is unknown [11, 12]. Therefore, it is difficult to determine whether this mutation is pathologically associated with the onset of aHUS.

In summary, we have described a patient who was diagnosed with STEC-HUS, which resulted in a pathology similar to that of acquired TTP. Our patient was successfully treated with the conventional supportive therapy. IgG autoantibodies against ADAMTS13 were not detected, which impaired our ability to diagnose clinical TTP. Furthermore, we identified a rare mutation of *MCP*, which has been associated with the onset of aHUS. It was difficult to determine whether this mutation was associated with disease in this patient, and we speculate that it may have led to exacerbation of disease. Notably, we were also unable to confirm a diagnosis of aHUS. This case was highly intriguing because of the involvement of distinct concurrent pathologies associated with TMA. In this case, STEC-HUS, DIC, reduced levels of non-IgG anti-ADAMTS13 autoantibodies, and the effect of an aHUS-like mutation combined to create a complicated pathological condition. Even in patients with apparently simple STEC-HUS, we consider it to be necessary to perform sufficient evaluation when healing is prolonged or unexpected clinical symptoms are observed.

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Compliance with ethical standards

Conflict of interest All the authors have declared no competing interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee at which the studies were conducted (IRB approval number Hi-204) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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