[CASE REPORT]

Streptococcal Toxic Shock Syndrome Induced by Group A Streptococcus with the *emm28* Genotype That Developed after a Uterine Cancer Test

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Abstract:

A 69-year-old woman without pre-existing disease visited our hospital due to general malaise, diarrhea, and arthralgia 3 days after a uterine cancer test. We diagnosed her with sepsis of unknown focus and started treatment immediately, but she died 20 hours after the first visit due to multi-organ failure and septic shock. Later, group A streptococcus was detected from the blood culture, and streptococcal toxic shock syndrome (STSS) was diagnosed. The strain had the *emm28* genotype and a mutation in *csrR* with increased NADase activity. These virulence factors were considered to be related to STSS development in this patient.

Key words: STSS, *emm*, *csrR*, NADase

(Intern Med 60: 3481-3483, 2021)

(DOI: 10.2169/internalmedicine.6290-20)

Introduction

In recent years, an increase in invasive group A streptococcus (GAS) infections has caused serious problems in various countries (1-5). Streptococcal toxic shock syndrome (STSS) has high fatality and occurs even in healthy individuals. Focal symptoms due to infection may become a feature of this severe disease, but primary symptoms are sometimes non-specific and interfere with the initial diagnosis. Skin is the most frequent clinical focus of this infection, but some cases involve infection of an organ other than the skin, and the site of entry cannot always be identified (1, 6).

Several virulence factors of GAS have been described recently (7). M protein encoded by *emm* protects GAS from phagocytosis by polymorphonuclear leukocytes (1), and the *emm* genotype is associated with the incidence of STSS. Furthermore, mutations in *csrS*, *csrR*, and *rgg* are associated

with STSS development (8). However, the relationship between these virulence factors and the actual clinical features is unclear.

We experienced a case of STSS caused by GAS with an *emm28* genotype, a mutation in *csrR*, and increased NAD+ glycohydrolase (NADase) activity.

Case Report

A 69-year-old woman without pre-existing disease visited our hospital complaining of general malaise, diarrhea, and arthralgia. She had undergone a screening test for uterine cancer three days before in another hospital, and abdominal pain and diarrhea subsequently developed. Her vital signs on arrival were as follows: blood pressure: 104/74 mmHg, heart rate: 115 beats/min, body temperature: 36.5°C, respiratory rate: 30 breaths/min, and oxygen saturation: 99% on room air. A physical examination conducted at that time revealed

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Received: September 12, 2020; Accepted: March 21, 2021; Advance Publication by J-STAGE: May 14, 2021

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Table 1. Laboratory Data on Admission.

Hematology		Biochemistry	
WBC	1,140 / μ L	TP	5.7 g/dL
Neut	76.3 %	Alb	3.5 g/dL
Lym	15.8 %	T-Bil	0.6 mg/dL
Mono	17.9 %	ALP	208 IU/L
Eos	0 %	AST	41 IU/L
RBC	379 $\times 10^4$ / μ L	ALT	22 IU/L
Hb	12.0 g/dL	LDH	475 IU/L
Plt	17.2 $\times 10^4$ / μ L	CK	619 IU/L
		BUN	37.0 mg/dL
Coagulation		Cre	1.90 mg/dL
PT-INR	1.13	Na	126 mEq/L
APTT	35.9 S	K	3.6 mEq/L
Fib	560 mg/dL	Cl	95 mEq/L
FDP	256.6 μ g/mL	Ca	7.3 mg/dL
AT-III	58 %	Glu	121 mg/dL
		PCT	58.4 ng/mL
Serology			
CRP	35.9 mg/dL		

generalized arthralgia. Abnormal findings suggestive of cellulitis or abscess were not found anywhere on her body, including the external genitalia. A gynecological pelvic examination was not performed.

The laboratory test results revealed a decreased white blood cell count, an increased C-reactive protein level, and renal failure (Table 1). No abnormal findings were found on chest X-ray, and abdominal computed tomography showed slight thickening of the intestinal wall and a small amount of ascites within the pelvis. We diagnosed her with sepsis of unknown origin, and she was hospitalized and started on antibiotic treatment (meropenem: 1,500 mg/day).

Her arthralgia subsequently worsened notably, and her blood pressure gradually decreased. The administration of noradrenaline was started, but her consciousness deteriorated further. She did not provide consent for invasive treatment, including intubation, and she died approximately 20 hours after the first visit due to multi-organ failure and septic shock. The next day, GAS was detected from the blood culture on admission. According to diagnostic criteria for STSS (9), she was diagnosed with STSS. The strain was not resistant to a series of antibiotics, including meropenem.

To investigate the virulence factors of GAS, we examined the *emm* genotype and the presence or absence of mutations in *csrR/csrS* and *rgg*. The methods used are shown in the Supplementary material. The strain from this patient had the *emm28* genotype, and mutations in *rgg* and *csrS* were not found. However, a single nucleotide change at the 62nd position (GAG→GGG) in *csrR* was found. We therefore quantified the NADase activity, which can be increased by a mutation in *csrR* (10, 11), and compared it with the values in strains derived from non-STSS patients. The methods used are shown in the Supplementary material. The NADase activity was higher than that of two *emm28* strains obtained from non-STSS patients and confirmed to lack *csrR/csrS*

Table 2. NADase (Nga) Activity of the Strain Obtained from This Case and Two *emm28* Strains without *csrR/csrS* Mutation from Non-STSS Patients.

Strain	Nga activity (U)
Strain obtained from this case	28.4 $\pm$ 5.6
13-T-23	0.84 $\pm$ 0.12
13-I-33	1.57 $\pm$ 0.35

13-T-23 and 13-I-33 strains were isolated from sputum and urine of non-STSS patients in Aichi Prefecture in Japan, respectively. The *emm* type of both strains was *emm28* determined as described in supplementary material. The *csrR/csrS* sequences of these strains were determined as described in supplementary material and identical to those of the most of the *emm28* strains in the genome database.

mutations (Table 2).

Discussion

STSS is a severe invasive infection mainly caused by GAS. It is characterized by the sudden onset of shock and multi-organ failure, and the mortality rate is 30%-70% despite antibiotics and supportive therapy (1). The most common symptom of STSS is pain, but an influenza-like syndrome characterized by a fever, chills, myalgia, and diarrhea appears in some patients (6). In the present case, severe diarrhea was the main symptom at the initial visit, which may have been a non-specific symptom associated with STSS. The criteria for the diagnosis of STSS have been previously published (9), but the diagnosis is often difficult because STSS may not show specific symptoms, as in our case. Clinicians should thus consider the possibility of STSS in any case with a rapid onset of shock without symptoms involving specific organs.

Several important virulence factors involved in GAS pathogenicity have been described (7). The M protein, a fimbrial surface protein encoded by *emm*, is a main factor. M protein inhibits the phagocytosis of GAS in the absence of opsonizing antibodies, promotes adherence to human epithelial cells, and helps the bacterium overcome innate immunity (12). A recent multicenter study analyzed 223 *emm* types of GAS (13). In the survey of strains obtained from STSS patients in Japan, the predominant genotype was *emm1*, followed by *emm89*, *emm12*, *emm28*, *emm3*, and *emm90*. These six genotypes constituted more than 90% of the STSS isolates (1). *emm1* is also the most prevalent *emm* genotype of STSS, which is in accordance with results from the USA, Japan, and across Europe (1, 2, 14). *emm28* is the most predominant genotype in gynecoid-obstetrical sepsis due to STSS (3), which is consistent with the susceptible site of entry of GAS in this case. To our knowledge, whether or not the *emm* genotype directly affects GAS pathogenicity has been unclear. An examination of the relationship between the *emm* genotype and GAS pathogenicity in strains

obtained from STSS patients is thus needed.

As in the present patient, cases of STSS triggered by a uterine cancer test, endometrial biopsy, or hysteroscopy have been reported (15, 16). Steven et al. reported that about 45% of STSS patients were infected by bacteria that invaded through the skin, about 20% of STSS patients were infected by bacteria that invaded through mucous membranes (pharynx, vagina), and the remaining roughly 35% involved an unknown entry site (6). In this case, we assumed that GAS in the vagina entered the blood due to a slight wound at the time of uterine cancer screening. Clinicians need to be aware that gynecological procedures are important to consider when obtaining a patient's history regarding STSS.

STSS isolates have a high frequency of mutations in two component regulatory system genes in GAS: *csrS* and *csrR*, and/or *rgg*, all of which are negative regulators of virulence factors. These mutations lead to the overproduction of multiple virulence factors regarded as factors responsible for STSS (8). In the strain obtained from the present patient, mutations were not recognized in *rgg* or *csrS*, but a single nucleotide change was noted at the 62nd position (GAG→GGG) in *csrR*, which resulted in a change in the 21st amino acid from glutamic acid to glycine. Mutations in *csrR* lead to increased NADase activity, which is a virulence factor of GAS (10, 11). The strain also showed an increase in NADase activity compared with strains obtained from non-STSS patients (Table 2). NADase activity seemed to be involved in the development of STSS in this case. In mouse models of invasive soft-tissue infection and septicemia, mutant GAS that lacked NADase activity had a significantly lower virulence than the isogenic wild-type parent, confirming the important role for NADase in the infection of a host animal (11).

In conclusion, we encountered a case of STSS due to *emm28* GAS that progressed rapidly. When examining patients with septic shock without an apparent infection focus, we should consider the possibility of STSS and conduct an endometrial test at that time if the patient is a woman. In addition, pathogenicity factors need to be further clarified in order to improve the treatment outcomes of this disease.

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

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