J Infect Chemother 25 (2019) 355–361

Contents lists available at ScienceDirect

Journal of Infection and Chemotherapy

journal homepage: http://www.elsevier.com/locate/jic

Original Article

Streptococcal toxic shock syndrome caused by β-hemolytic streptococci: Clinical features and cytokine and chemokine analyses of 15 cases☆

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ARTICLE INFO

Article history:
Received 27 September 2018
Received in revised form
28 December 2018
Accepted 13 January 2019
Available online 7 February 2019

Keywords:
Streptococcal toxic shock syndrome
Pathogenesis
Cytokines
Chemokines

ABSTRACT

Objectives: β-Hemolytic streptococci occasionally cause severe infections such as necrotizing fasciitis and streptococcal toxic shock syndrome (STSS). Here, we conducted a prospective study to investigate the production of cytokines and chemokines in patients with STSS to explore its pathogenesis in survivors and fatal cases.

Methods: From January 2013 through August 2015, all culture results from normally sterile sites were prospectively followed and screened for STSS. Clinical characteristics of the patients with STSS were evaluated and compared between survivors and fatal cases. Serum samples were collected on admission for quantification of various cytokines and chemokines. Bacterial strains were categorized by Lancefield grouping and analyzed for the *emm* type, and presence of *speA*, *speB*, *speC*, and *speF*.

Results: Fifteen patients received diagnosis of STSS. The median age of the patients was 60-year-old, and the mortality rate was 40% despite intensive treatment. Nine strains were categorized as group A, two belonged to group G, and four to group B. Group A contained various *emm* genotypes. Unexpectedly, potent proinflammatory cytokine levels such as TNF-α and IL-1β were not significantly elevated, and comparison with surviving patients showed that IL-6, IL-8, and MCP-1 levels were significantly decreased and creatine kinase level was significantly elevated in fatally ill cases.

Conclusion: Our results indicate that reduced production of proinflammatory cytokines and chemokines may be involved in STSS pathogenesis and critical for prognosis of patients with STSS.

1. Introduction

Streptococcal toxic shock syndrome (STSS) is a notorious infectious disease, which is characterized by abrupt onset and rapid progression with high mortality rate. The case fatality rate of STSS is 35–45%, which is almost twice that reported for invasive group A *Streptococcus* (GAS) cases lacking shock manifestation [1–5]. Most patients die within a week of developing the symptoms despite aggressive and intensive treatment [6]. In Japan, since the first case of STSS was reported in 1993 [7], almost 100 patients have been reported to have STSS due to GAS infection according to the National Institute of Infectious Diseases and prefectural Public Health Institutes Surveillance in Japan [8].

GAS is the most common microorganism that causes STSS; however, recently, other β-hemolytic streptococci such as group B, C, and G streptococci were also reported to cause a similar clinical course [5,9–12]. The pathogenesis of STSS has been elucidated mostly in terms of virulence factors of bacterial strains, such as M protein, superantigens, and pyrogenic toxins [1,10,13–16]. Many streptococcal virulence factors have been implicated in the pathogenesis of streptococcal infections,

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https://doi.org/10.1016/j.jiac.2019.01.006
1341-321X/

Table 1
Clinical and laboratory features of the cases.

Case No.	Age	Sex	Comorbidity	Strain isolated from sterile site	Focus	History of injury within 1 week	Cellulitis/Erythema	Purpura	Necrotizing fascitis	ARDS	DIC	GCS	Days from onset to hospital visit
1	68	F	SLE	GAS(S. pyogenes)	RLE	+	+	+	+	−	+	E1V1M1	3
2	60	F	unknown	GAS(S. pyogenes)	BLE	unknown	+	+	+	−	+	E1V2M5	unknown
3	89	F	CHF	GAS(S. pyogenes)	RLE	+	+	+	+	−	+	E4V5M6	4
4	52	M	LC, DM	GAS(SDSE)	RLE	+	+	−	−	+	+	E3V4M5	1
5	34	F	none	GAS(S. pyogenes)	meningitis	−	−	−	−	−	+	E1V1M5	5
6	68	M	RA	GAS(S. pyogenes)	LUE	−	+	+	+	+	+	E4V5M6	5
7	67	F	breast cancer on chemotherapy	GAS(S. pyogenes)	LLE	+	+	−	+	−	+	E3V5M6	1
8	41	M	none	GAS(S. pyogenes)	RLE	+	+	+	+	−	+	E4V5M6	3
9	65	M	stroke	GGS(SDSE)	RLE	−	+	+	+	−	+	E3V5M6	5
10	83	M	stroke	GGS(SDSE)	unknown	−	+	−	−	−	+	E3V5M6	1
11	54	M	LC, hemophilia	GBS(S. agalactiae)	bilateral knee	−	+	−	−	−	+	E3V4M6	6
12	81	F	CRF(HD)	GBS(S. agalactiae)	RLE	+	+	+	+	−	+	E4V5M6	1
13	56	M	LC, DM	GBS(S. agalactiae)	RLE	unknown	−	−	−	−	+	E1V2M4	unknown
14	46	M	DM	GAS(S. pyogenes)	Lung	−	−	−	−	−	+	E4V5M6	4
15	56	M	LC, Wilson disease	GBS(S. agalactiae)	RLE	−	+	+	+	−	+	E4V5M6	1
Median	60												3

NA: not applicable, GAS: group A Streptococcus, RLE: right lower extremities, DIC: disseminated intravascular coagulation, MEPM: Meropenem, CEZ: Cefazolin, WBC: white blood cell, CHF: chronic heart failure, GGS: group G Streptococcus, BLE: bilateral lower extremities, GCS: Glasgow Coma Scale, PCG: Penicillin G, SBT/ABPC: Sulbactam/Ampicillin, Cr: creatinine, LC: liver cirrhosis, GBS: group B Streptococcus, LUE: left upper extremities, ARDS: acute respiratory distress syndrome, CLDM: Clindamycin, VCM: Vancomycin, DM: diabetes mellitus, SDSE: Streptococcus dysgalactiae subspecies equisimilis, LLE: left lower extremities, LZD: Linezolid, DRPM: Doripenem, NA: not applicable, RA: rheumatoid arthritis, CTRX: Ceftriaxone, TAZ/PIPC: Tazobactum/Piperacillin, CRF: chronic renal failure, HD: hemodialysis, AZM: Azithromycin.

including the M protein encoded by the *emm* gene. The M protein protects GAS from phagocytosis by polymorphonuclear leukocytes [17,18]. A recent multicenter study analyzed 223 *emm*-types of GAS [19]. Most queries will obtain an exact 180/180 match to one of the database entries that can be assigned to the sequence (e.g., emm4.4 is equivalent to type emm4, subtype emm4.4). The subtype has been correctly identified if a perfect 180/180 match is obtained from the type-specific BLAST. The decimal point indicates a subtype. Recently, Norrby-Teglund et al. provided evidence regarding the mechanism of host immune response to STSS based on in vivo and in vitro studies, indicating possible immunogenetic differences in the outcomes of invasive group A streptococcal infections on the basis of the proportion of cytokine-producing cells among peripheral blood mononuclear cells (PBMCs) [16,20–23]. In contrast, only few have assessed circulating cytokine profiles in patients with STSS. Here, we conducted a prospective study to explore the pathogenesis of STSS in terms of bacterial and host factors by investigating the clinical features, isolated bacterial strains, and circulating cytokine and chemokine profiles of patients with STSS.

2. Materials and methods

2.1. Setting

The Toho Medical Center Omori Hospital is a comprehensive tertiary care teaching hospital in Tokyo with 936 beds and more than 800,000 patient visits per year.

2.2. Patients

From January 2013 through August 2015, all culture results from normally sterile sites were prospectively followed and screened for STSS according to the STSS definition as previously reported [24]. The patients were evaluated for their clinical characteristics (e.g. days from onset to admission, history of injury, comorbidities, complications, physical findings, laboratory data, treatment, and prognosis) and comparison of the clinical characteristics between survivors and fatal cases was executed.

2.3. Bacterial isolates

β-Hemolytic streptococci isolated from normally sterile sites of patients with STSS were analyzed for Lancefield grouping, serotype, *emm* type, and presence of *speA*, *speB*, *speC*, and *speF*. The antibiotic sensitivity of the isolates was determined by the micro broth dilution method according to the Clinical and Laboratory Standards Institute guidelines.

2.4. emm typing

emm sequencing was performed as described by Beall et al. [25] with modifications described at https://www.cdc.gov/streplab/index.html. According to the CDC protocol, the primers emm-1 (5′-TATT(C/G)GCTTAGAAAATTAA-3′) and emm-2 (5′-GCAAGTTCTTCAGCTTGTTT-3′) were used for amplifying the N-terminal region of *emm*. The purified PCR products were sequenced using the emmseq2 primer (5′-TAA TCG CTT AGA AAA TTA AAA ACA GG-3′). The first 160 bases of the 5′-end of *emm* were compared to those in the CDC *emm* sequence database (https://www2a.cdc.gov/ncidod/biotech/strepblast.asp).

2.5. Detection of virulence genes by polymerase chain reaction (PCR)

PCR with previously described primer pairs was conducted for detecting genes encoding streptococcal pyrogenic exotoxins (*speA*, *speB*, *speC*, and *speF*) [25,26]. In all cases, the primers were designed for a sequence located inside an open reading frame. The expected sizes of amplicons were 393 bp for *speA*, 1113 bp for *speB*, 540 bp for

Days from admission to death	Antibiotic Treatment	Prognosis (30 day mortality)	Body temperature °C	WBC /μL	CRP mg/dL	Platelet ×10^4/μL	Glucose mg/dL	CK U/L	Cr mg/dL	AST U/L	HbA1c
2	MEPM + CLDM	dead	35.7	30,800	19.8	7.7	7	3028	5.0	174	NA
3	DRPM + ABPC + CLDM + VCM	dead	34.6	500	11.7	10.2	37	1627	1.5	105	5.2
2	SBT/ABPC + CLDM	dead	37.3	1200	18.3	14.9	85	2692	2.1	105	5.2
23	MEPM → PCG + CLDM	dead	40.5	2400	11.4	4.3	119	1845	1.0	159	8.6
25	MEPM + LZD + CLDM → CTRX + CLDM	dead	40.6	16,500	19.9	15.5	164	750	0.5	300	6.1
6	CEZ + CLDM	dead	35.3	29,200	29.2	31	147	816	3.7	32	5.6
NA	SBT/ABPC + CLDM	alive	36.9	1200	16.3	23.4	83	131	1.3	40	5.5
NA	MEPM + VCM + CLDM → SBT/ABPC + CLDM	alive	38.7	2300	22	12.4	118	181	4.0	39	5.1
NA	MEPM + VCM → PCG + CLDM	alive	38.2	6000	37.7	11.7	117	768	4.8	55	5.5
NA	MEPM + VCM → ABPC + CLDM	alive	38.2	13,300	0.7	10.2	142	156	1.6	21	NA
NA	DRPM → SBT/ABPC	alive	36.7	11,500	12.4	5.9	114	21	2.3	64	5.2
NA	SBT/ABPC + CLDM	alive	38.2	9300	13.3	8.3	77	56	4.75(HD)	24	NA
NA	TAZ/PIPC + CLDM→ SBT/ABPC	alive	25.8	5700	16.4	12	21	1112	0.9	214	7.6
NA	MEPM + VCM + AZM → TAZ/PIPC + CLDM → SBT/ABPC	alive	37.8	10,000	34.9	8.1	809	255	3.7	23	11.2
NA	MEPM + VCM + CLDM → SBT/ABPC	alive	36.6	14,800	3.3	6.7	90	91	2.7	401	4.6
5		mortality 40%	37	9300	16	10	114	750	2	64	6

speC, and 238 bp for *speF*. PCR amplification was performed by initial denaturation at 95 °C for 10 min, followed by 30 cycles of 95 °C for 30 s, 52 °C for 30 s, and 72 °C for 2 min, and a final extension at 72 °C for 3 min.

2.6. Cytokine analysis

Serum samples of patients with STSS were collected on admission and a variety of cytokines (IFN-γ, TNF-α, IL-1β, IL-6, IL-12 p70, IL-17A, G-CSF, IL-10, IL-4, and IL-2) and chemokines (MCP-1, IP-10, IL-8) were quantified using Flow Cytomix (eBioscience) on a FACSCalibur flow cytometer (Becton, Dickinson and Company). Limits of detection (pg/mL) were as follows: IFN-γ, 1.6; TNF-α, 3.2; IL-1β, 4.2; IL-6, 1.2; IL-12 p70, 1.5; IL-17A, 2.5; G-CSF, 3.4; IL-10, 1.9; IL-4, 20.8; IL-2, 16.4; MCP-1, 2.2; IP-10, 6.0; and IL-8, 0.5. Comparison of the immunological responses between survivors and fatal cases were executed.

2.7. Statistical analysis

These analyses were performed in the GraphPad Prism 5 software (GraphPad, Inc., La Jolla, CA). Continuous comparisons were made using the Mann–Whitney test. Differences were considered statistically significant at $p < 0.05$.

2.8. Ethics statement

This study complied with the guidelines of the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. The protocol was approved by the Institutional Individual Ethics Committee (Toho Medical Center Omori Hospital Ethic Review Board, Decision no. 27–128). Informed consent was obtained from all the individual participants of the study.

3. Results

3.1. Clinical characteristics of patients with STSS

In total, 19,693 samples were obtained from 6249 patients, and 1308 patients showed positive culture results at normally sterile sites. Among these, 15 cases were diagnosed as STSS because of the presence of β-hemolytic streptococci. The median age of these patients was 60 years (range 34–89 years); four patients had liver cirrhosis and three patients had diabetes mellitus. Six patients had a history of injury or contusion within a week of the hospital visit. The median number of days from the disease onset to the hospital visit was three, and the mortality rate was 40%, despite intensive treatment such as aggressive antibiotic therapy, immunoglobulin and vasopressor administration, and continuous hemodiafiltration. All the cases presented as bacteremia, and 12 patients had cellulitis in lower extremities, of which nine patients had necrotizing fasciitis. Body temperature on admission varied from very low to high (25.8–40.6 °C, median 37.0 °C). All patients had disseminated intravascular coagulation, and two patients had acute respiratory distress syndrome. Although both very low and high white blood cell (WBC) counts were observed, the median white blood cell (WBC) count was 9300 cells/L (range 500–30,800 cells/L; Table 1). To investigate the clinical differences between surviving patients and fatal cases, body temperature, WBC counts, C-reactive protein (CRP), platelets, glucose, creatine kinase (CK), creatinine, and aspartate transaminase (AST) levels were compared. Only CK levels were statistically significantly elevated among fatal cases (the median value of fatal cases: 1736 U/L, surviving patients: 156 U/L, $p = 0.0004$); no other significant differences were noted (Table 2).

3.2. Features of bacterial isolates

Nine strains were categorized in group A (all isolates were *S. pyogenes*, except for one strain identified as *S. dysgalactiae*

Table 2
Comparison of the clinical characteristics between survivors and fatal cases.

	Survivors (n = 9)	IQR	Fatal outcome (n = 6)	IQR	p value[a]
Age (year)	56	54–67	64	54–68	0.3839
Male/Female	7/2	NA	2/4	NA	0.1357
Body Temperature(C)	37.8	36.7–38.2	37.3	35.7–40.5	0.4296
WBC (×10^3 cells/L)	9.3	5.7–11.5	9.45	1.5–26.0	0.4068
Plt (×10^4/μL)	10.2	8.1–12	12.6	8.3–15.4	0.3185
CRP (mg/L)	16.3	12.4–22	19.1	13.4–19.9	0.432
Cr (mg/dL)	2.5	1.5–3.8	1.8	1.1–3.3	0.3026
AST (U/L)	40	24–64	64	40–105	0.0875
CK (mg/dL)	156	91–255	1736	1019–2480	0.0004

Data are shown as median.
IQR: Interquartile Range.
NA: not applicable.
[a] Mann Whitney test, except for gender analysis (Fisher's exact test).

subspecies *equisimilis*), two belonged to group G (all isolates were *S. dysgalactiae* subspecies *equisimilis*), and four belonged to group B (all isolates were *S. agalactiae*). In group A, there were three *emm12.0* and two *emm1.0* strains each. Other strains belonged to different subtypes. Among eight *S.pyogenes*, two strains tested positive for *speA*, and eight and seven strains tested positive for *speB* and *speF*, respectively. Five strains were positive for *speC*. Serotypes of groups B differed from one another, except for two strains, which had the Ib type (Supplementary Table 1). All isolates were sensitive to penicillin G (MIC < 0.06 μg/mL), cefotaxime, meropenem, vancomycin, and linezolid. Six isolates were resistant to erythromycin and four isolates were resistant to clindamycin (Supplementary Table 2).

3.3. Cytokine and chemokine analyses of the cases

The serum cytokine and chemokine levels were analyzed for fourteen patients except for one fatal case, the serum sample of which was not available. Unexpectedly, potent proinflammatory cytokines such as TNF-α and IL-1β were not significantly elevated except for patient No.2, who was a homeless and found lying on the ground so the precise clinical course was not known. On the other hand, relatively high levels of IL-6, IL-8, and G-CSF (median 6566.4, 2212.6, and 2420.2 pg/mL, respectively) and high concentrations of MCP-1 and IP-10 were detected (11,065.9 and 3122.4 pg/mL, respectively; Supplementary Table 3). Furthermore, the cytokine and chemokine levels were statistically compared to identify the immunological features of the surviving and fatally ill patients. IL-6, IL-8, and MCP-1 were significantly decreased among fatal cases than in surviving patients (Fig. 1). Other cytokine and chemokine levels were not significantly different between the two groups.

4. Discussion

It is well-known that bacterial sepsis induces proinflammatory cytokines such as IL-6 and TNF-α; however, reports on immunological and clinical features of patients with STSS are limited. Here, we identified the clinical and bacterial features of such patients, including novel data from cytokine and chemokine analyses.

Bacterial sepsis causes both hyperinflammation and immunosuppression, which sometimes occur simultaneously. TNF-α, IL-1β, and IL-6 are the cytokines that mediate the initial response of innate immunity to injury or infection. TNF-α and IL-1β both activate endothelial cells, attracting circulating polymorphonuclear leukocytes to the infection site. They also enter the circulation, causing fever and other systemic symptoms. IL-6 enhances hepatic production of the acute phase reactants, including CRP, and stimulates a shift in the production of immune cells in the bone marrow to induce formation of more polymorphonuclear leukocytes. Therefore, these three cytokines are essentially responsible for the features of systemic inflammatory response syndrome [27,28]. The common chemokines, IL-8, IP-10, and MCP-1 are often as reliable as IL-6 at measuring the magnitude of an injury or sepsis response [29,30]. Bozza et al. have shown that IL-8 and MCP-1 have the best accuracy for predicting 28-day mortality, while IL-6 and IL-8 are good predictors of worsening organ dysfunction in patients with severe sepsis [31]. In a prospective pilot study of 29 patients, Anuluz-Ojeda et al. recently showed that IL-6, IL-8, IL-10, and MCP-1 levels were elevated in patients who later died compared to those who survived severe sepsis [32]. Several reports mention cytokine and/or chemokine analysis of STSS in experimental models or patients. Saito et al. studied a mouse model of *S. pyogenes*-induced STSS [33]. The mice were inoculated with 10^7 colony-forming units of *S. pyogenes* intramuscularly, and cytokine levels were measured immediately before death. Relatively high concentrations of TNF-α, IFN-γ, IL-6, and MCP-1 were observed, but most mice died almost 20 days after bacterial inoculation [33]. Stevens et al. reported high levels of TNF-α in a baboon model of *S. pyogenes* bacteremia with profound hypotension, where administration of a neutralizing anti-TNF-α antibody restored normal blood pressure and reduced mortality by 50% [34]. Another report showed the cytokine profile of children with *S. pyogenes* infection could be stratified by severity of the disease [35]. Patients with invasive GAS disease had significantly higher IFN-γ, IL-1β, IL-6, IL-8, IL-10, and IL-18 levels than those with non-invasive diseases or colonization and healthy controls. There was no difference in TNF-α, IL-12, and IL-2 levels among the groups [35]. Another study on serum TNF-α levels of three patients with STSS showed that TNF-α was not upregulated in any of the patients [36]. Compared to the results of previous studies, our findings obtained by comparing the characteristics of surviving patients and fatal cases, are novel. Unexpectedly, our results indicate that low TNF-α levels were detected among survived and fatal cases (Supplementary Table 3). Furthermore, IL-6, IL-8, and MCP-1 concentrations were significantly lower among the fatal cases than in the surviving patients (Fig. 1). These results suggest that a relatively weaker response of the host to the pathogen may affect the prognosis. In contrast, several reports discuss the role of inflammatory cytokines in invasive streptococcal infections, which demonstrate that patients with a propensity to produce higher levels of inflammatory cytokines in response to streptococcal superantigens develop significantly more severe systemic manifestations than patients who tend to produce lower levels of inflammatory cytokines to the same superantigens. These findings suggest that host factors influence the magnitude of cytokine response to superantigens and consequently the clinical outcome of the infection [20]. Further study demonstrated that patients with the DRB1*1501/DQB1*0602 haplotype mounted significantly lower responses and were less likely to develop severe systemic disease,

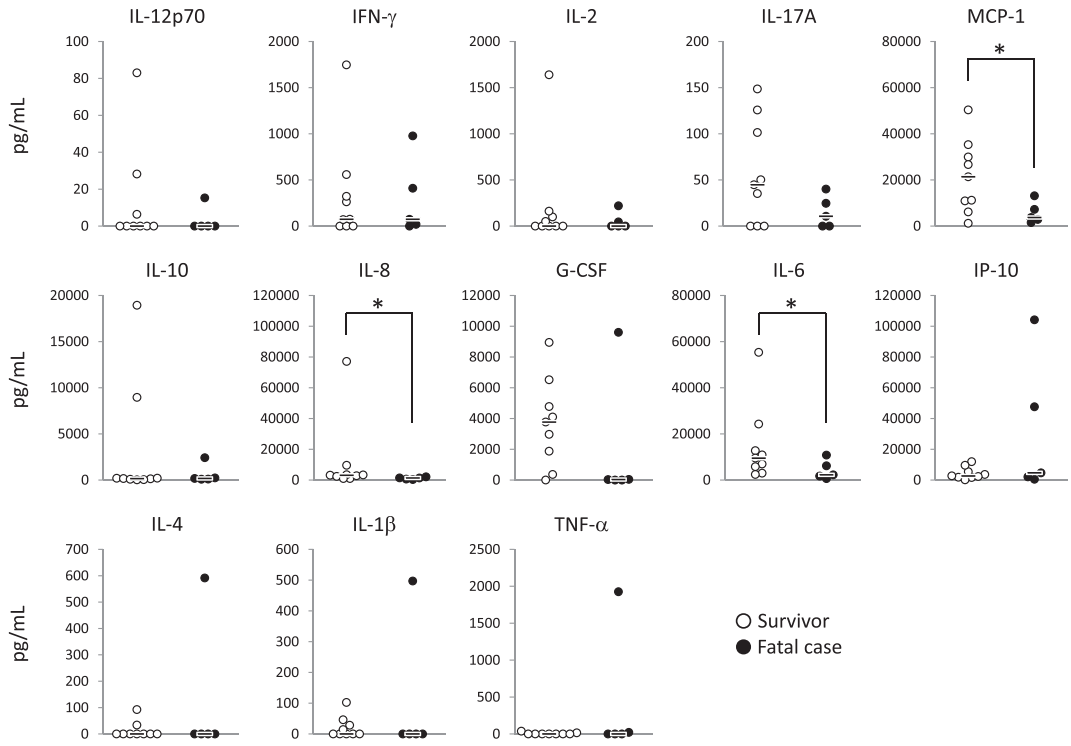

Fig. 1. Cytokine and chemokine profiles of 14 patients with STSS. Serum cytokine and chemokine levels were determined by flow cytometry analysis. Bars indicate the median. The Mann-Whitney test shows statistically significant differences between survivors and fatal cases (*$P < 0.05$).

suggesting that human leukocyte antigen class II allelic variation contributes to differences in severity of invasive streptococcal infections via their ability to regulate cytokine responses triggered by streptococcal superantigens [21]. The differences between our findings and previous results lie mainly in the method used for investigating cytokine production. Norrby-Teglund et al. analyzed cytokine production by enumerating the number of cytokine-producing cells among PBMCs using Golgi-confined stain as a marker, whereas we detected circulating cytokines in serum by flow cytometry. We measured both plasma and serum cytokine levels for some of the patients, which did not alter the results (data not shown). We decided to analyze serum cytokine levels similar to former reports [33,34] because of practical convenience.

Cytokines generated early during the infection may be important for controlling bacterial dissemination, although excessive and persistent production of cytokines may be detrimental [37]. A report shows selective depletion of V β-bearing T cells in patients with severe invasive GAS infections and STSS. V β-bearing T-cell depletion was associated with extensive DNA fragmentation, suggesting that one of the mechanisms underlying this depletion might be apoptosis [38]. Our findings indicate reduced immunological response among fatally ill patients, which may provide clues regarding its pathogenesis. Further studies are required to precisely characterize the pathogenesis of STSS.

In terms of the bacterial features of our cases, no specific serotype, *emm* type, or superantigen pattern was prevalent except for the detection of two strains with the T1/M1 serotype and four strains with the T12/M12 serotype.

Our study is not without limitations. First, cytokines such as TNF-α have short half-life and the response is phasic. Therefore, cytokine quantification may not have been conducted at the best time point. Patients visited hospitals at various time points relative to the onset; however, most of them visited shortly after the onset of symptoms. The median time for hospital visit was three days. Therefore, it may be assumed that serum cytokines were quantified relatively early in the clinical course. Certain patients were able to provide serum samples at several time points, which enabled determination of the time course of cytokines production (data not shown). Second, we compared the clinical characteristics and cytokine/chemokine profiles among survivors and fatal cases. Patients may die early in the course due to excessive inflammation and organ dysfunction; however, a significant proportion die later in the course [39], which is not related to excessive inflammation. All fatal cases observed in this study died within 30 days, with median time from admission to death being 5 days as shown in Table 1. These are considered as cases of early death, and may be used for comparisons between survivors and fatal cases.

5. Conclusions

In summary, we demonstrated clinical characteristics and cytokine and chemokine profiles of 15 patients with STSS. Most of the cases were caused by different serotypes of β-hemolytic streptococci, except for two strains of T1/M1 and four strains of T12/M12. Overall, potent proinflammatory cytokines such as TNF-α, IL-1β, and IFN-γ were not significantly elevated, whereas relatively high concentrations of IL-6, IL-8, and G-CSF were observed. Unexpectedly, in a comparison of surviving patients and fatal cases, IL-6, IL-8, and MCP-1 were found to be significantly decreased and a relatively high concentration of IP-10 was detected among fatal cases. Our results indicate that reduced production of proinflammatory cytokines such as IL-6 and certain chemokines such as IL-8 and MCP-1 may be involved in STSS pathogenesis. Further studies may provide insights into novel strategies against the complicated pathophysiology of STSS.

Funding

This study is partially supported by Japan Agency for Medical Research and Development (AMED), Japan under Grant Number

JP18fk0108044 (TM and TI) and Japan Society for the Promotion of Science (JSPS) KAKENHI, Japan Grant Numbers JP25460085 and JP16K09952 (TM).

Conflicts of interest

The authors declare that they have no competing interests to this study.

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Acknowledgements

We thank Dr. Katsunori Yoshihara from the Department of Emergency and Critical Care Center, Toho University Omori Medical Center, for assistance with regulatory coordination and clinical data collection, as well as Dr. Keizo Yamaguchi, Professor Emeritus of the Department of Microbiology and Infectious Diseases, Toho University Faculty of Medicine, for special supervision. We are also grateful to the clinical microbiology staff at Toho University for their assistance with collecting the bacterial isolates.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jiac.2019.01.006.

List of abbreviations

STSS	streptococcal toxic shock syndrome
TNF-α	tumor necrosis factor-α
IL	interleukin
IP	interferon-gamma-induced protein
MCP	monocyte chemotactic protein
G-CSF	granulocyte colony-stimulating factor
GAS	group A *Streptococcus*
PBMC	peripheral blood mononuclear cell
PCR	polymerase chain reaction

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