International Journal of Infectious Diseases (2004) **8**, 292–298

ELSEVIER

FOR INFECTIOUS DISEASES

http://intl.elsevierhealth.com/journals/ijid

Detection of circulating superantigens in an intensive care unit population☆

Kazuaki Azuma[a], **Kaoru Koike**[b,*], **Tatsuho Kobayashi**[a], **Toru Mochizuki**[a], **Kunihiro Mashiko**[a], **Yasuhiro Yamamoto**[c]

[a] *Department of Emergency and Critical Care Medicine, Chiba-Hokuso Hospital, Nippon Medical School, Chiba, Japan*
[b] *Department of Emergency and Critical Care Medicine, Tohoku University, Graduate School of Medicine, Miyagi, Japan*
[c] *Department of Emergency and Critical Care Medicine, Nippon Medical School, Tokyo, Japan*

Received 25 February 2003; received in revised form 7 November 2003; accepted 11 December 2003
Corresponding Editor: Jonathan Cohen, Brighton, UK

KEYWORDS
Superantigens;
Gram-positive bacteria;
Sepsis;
Septic shock;
Enzyme-linked immunosorbent assay;
Intensive care unit

Summary *Objective:* Plasma concentrations of superantigens were measured in an intensive care unit (ICU) population and the relationship of superantigen positive rates with the presence of sepsis was investigated.

Methods: Plasma samples were collected at least twice a week from 78 patients whose primary diagnoses were abdominal disorders ($n = 27$), respiratory disorders ($n = 11$), trauma ($n = 10$), burns ($n = 10$), cardiovascular disorders ($n = 4$), neurological disorders ($n = 2$), and others ($n = 14$). Five different species of superantigens, i.e., staphylococcal enterotoxins A, B, and C (SEA, SEB, and SEC), toxic shock syndrome toxin-1 (TSST-1), and streptococcal pyrogenic exotoxin A (SPEA), were measured using an enzyme-linked immunosorbent assay.

Results: Significant levels of plasma superantigens were detected in 16 patients. SEA was found in seven patients, SEB in four patients, SEC in two patients, TSST-1 in six patients, and SPEA in five patients. Superantigen detection rates were 6% (1/17) in patients without systemic inflammatory response syndrome (SIRS), 0% (0/21) in SIRS patients without infection, 31% (5/16) in septic patients without shock, and 42% (10/24) in septic shock patients.

Conclusions: The presence of superantigens was confirmed in part of the ICU population. The role of superantigens in the pathogenesis of sepsis remains to be determined.

Introduction

Sepsis is a major cause of death in patients admitted to intensive care units (ICUs), and the mortality from septic shock remains unacceptably high at 35–45%.[1] It has generally been considered that sepsis associated with Gram-negative bacterial

☆ This article is based on a study first reported in the Journal of the Japanese Association for Acute Medicine 2002;**13**:239–45.

*Corresponding author. Tel.: +81 22 717 7489; fax: +81 22 717 7492.
E-mail address: kkoike@gonryo.med.tohoku.ac.jp (K. Koike).

1201-9712/$30.00
doi:10.1016/j.ijid.2003.12.005

infections is more serious than that with Gram-positive infections. Recent evidence, however, suggests that the mortality from Gram-positive sepsis is comparable with or even greater than that from Gram-negative sepsis.[2] Furthermore, involvement of both Gram-positive and Gram-negative pathogens is frequently observed in a considerable number of cases with septic shock.[3]

Recent studies have focused on the difference between the pathogenesis of Gram-positive sepsis and that of Gram-negative sepsis[4–6] and have suggested the important role of superantigens in the pathophysiological mechanism of sepsis. A number of toxins produced by Gram-positive bacteria have been identified as superantigens. Such toxins include staphylococcal enterotoxins (SEs: SEA, SEB, SEC1-3, SED, SEE, SEG, SEH, SEI, and SEJ), toxic shock syndrome toxin-1 (TSST-1), streptococcal pyrogenic exotoxins (SPEs: SPEA, SPEC, SPEG, SPEH, SPEJ, and SPEL), and streptococcal mitogenic exotoxin Z1-2.[7–9] These superantigens have been shown to be potent activators of the immune system and to cause a variety of diseases in humans, ranging from food poisoning to toxic shock syndrome (TSS).[10,11]

Unlike conventional antigens, superantigens are known to activate T cells through binding to the class II major histocompatibility complex (MHC) on antigen presenting cells (APCs) without undergoing the intracellular processing pathway.[12] Furthermore, superantigens have been shown to bind to the β chain variable region (Vβ) of T cell receptor (TCR) molecules.[13] These interactions result in excessive production of proinflammatory cytokines and T cell proliferation linking to the development of clinical symptoms such as fever and hypotension.[14,15] In addition, superantigens have been shown to act synergistically with lipopolysaccharide to produce lethal shock.[16] Urine and plasma levels of TSST-1 and SPEA have been measured in TSS patients.[17–19]

The accumulating evidence suggesting the important role of superantigens in the pathophysiology of sepsis prompted the examination of actual plasma levels of superantigens in patients admitted to ICUs. In this report, plasma levels of SEA, SEB, SEC, TSST-1, and SPEA in an ICU population are measured and the relationship between the superantigen positive rate and the presence of sepsis is determined.

Materials and methods

Patients and blood sampling

This study was conducted in the medical and surgical intensive care units (ICUs) of Chiba-Hokuso Hospital and Sendagi Hospital, Nippon Medical School. They also function as level 1 trauma centers. Blood samples were obtained from 22 consecutive septic patients who were admitted to the ICU of Chiba-Hokuso Hospital between October 1997 and January 1999, and from 56 consecutive patients admitted to the ICU of Sendagi Hospital between October 1998 and February 1999. Patients with cancer, immunodeficiency, a cardiovascular disorder such as coronary disease, or a serious chronic disease were excluded from the study. The diagnosis of systemic inflammatory response syndrome (SIRS), sepsis and septic shock was determined, based on the criteria recommended by the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference Committee.[20] Blood sampling from each patient was performed at least twice a week. The collected blood samples were centrifuged at 3000 rpm and 4 °C for 15 mins. Each plasma sample was divided into aliquots, then immediately frozen, and kept at −80 °C until use. When an increase in body temperature, white blood cell count, or C-reactive protein level was detected, cultures of blood, pulmonary aspirate, urine, and/or other sites of possible sources of infection were obtained.

The enzyme-linked immunosorbent assay used in this study for determining plasma levels of superantigens was expected to be sensitive enough and to require no extra blood sampling except for routine examination for hematology and clinical chemistry. Considering these conditions, the Ethics Committee on Research at both hospitals concluded that it was unlikely that patients would be exposed to additional risk due to their enrollment. The committee also confirmed that the treatment and management of the enrolled patients was completely independent of the results of the serological screening. Under these circumstances, the committee concluded that it was not necessary to obtain written informed consent from each patient, but recommended that the patients should be provided with sufficient information about this study by the investigators.

Measurement of plasma concentrations of superantigens

Plasma concentrations of SEA, SEB, SEC, TSST-1 and SPEA were measured at a single laboratory by enzyme-linked immunosorbent assay (ELISA; Toray Industries Inc., Tokyo, Japan).[21] In brief, plasma samples were diluted two-fold and placed in wells of an ELISA plate precoated with polyclonal antibodies against a superantigen. Each plate was treated with a horseradish

peroxidase-labeled monoclonal antibody against the superantigen and then with the substrate, 3,3′,5,5′-tetramethyl-benzidine (Katayama Chemical, Osaka, Japan). The enzyme reaction was terminated by the addition of 0.1 mL of 0.5 M sulfuric acid, and the ELISA plates were read spectrophotometrically at 450 nm. Each of the superantigens, SEA, SEB, SEC, TSST-1 and SPEA, were subjected to a series of dilutions with buffer containing 2.5% bovine serum albumin (Seikagaku Corporation, Tokyo, Japan) and used as standard solutions for obtaining a calibration curve. The quantitation limits for SEA, SEB, SEC, TSST-1 and SPEA were 1.4, 5.9, 16.3, 2.5 and 4.3 pg/mL, respectively, in buffer containing 50% human plasma. A confirmatory experiment with purified samples of SEA, SEB, SEC, TSST-1 and SPEA indicated no cross-reaction between any pair of the superantigens at a concentration of 800 pg/mL in the ELISA. Additionally, purified staphylococcal protein A and streptococcal protein G did not react with the ELISAs at a concentration of 1 mg/ml, because the Fc fragment of monoclonal antibodies was digested by pepsin and the IgG fraction from rabbit and mouse sera was supplemented in the dilution buffer.[21] The specificity of ELISAs for staphylococcal toxins was also confirmed by comparing the data from reverse passive latex agglutination assay (Denkaseiken, Tokyo, Japan).[21] For evaluating ELISA results, a superantigen positive response was defined as superantigen levels higher than the respective 99% cut-off values, at which the chance of giving a positive ELISA response in a healthy patient is less than 1%. The 99% cut-off concentrations of SEA, SEB, SEC, TSST-1 and SPEA were 6, 50, 70, 25 and 10 pg/mL, respectively.

Statistical analysis

The relationship between the superantigen positive rate and the severity of infection was analyzed by the Ryan's test for multiple comparison of proportions[22] without any stratification by the sample number from each patient or by primary diagnosis.

Results

Plasma superantigen levels were examined in 78 ICU patients (60 males and 18 females) in total. These patients were 18—83 years old (median: 52) and primarily diagnosed with abdominal disorders ($n = 27$), respiratory disorders ($n = 11$), trauma ($n = 10$), burns ($n = 10$), cardiovascular disorders ($n = 4$), neurological disorders ($n = 2$), and others ($n = 14$) (Table 1). Significantly positive responses were observed in 34/474 (7%) of the plasma samples obtained. The superantigen positive samples were derived from 16 patients who had primary diagnoses of cardiovascular disorders ($n = 1$), respiratory disorders ($n = 3$), abdominal disorders ($n = 9$) [gastrointestinal ($n = 5$) and others ($n = 4$)], trauma ($n = 1$), and burns ($n = 2$). The percentage of superantigen positive patients among each primary diagnosis group ranged from 0 to 44% and showed no apparent correlation between the positive rates and primary diagnosis.

From the 16 superantigen positive patients, a total of 203 plasma samples were obtained and only 17% of the samples demonstrated significantly positive responses (Table 2). The percentage of

Table 1 The numbers of patients and plasma samples studied and the positive rates for at least one species of superantigens.[a]

Primary diagnosis	Number of patients	Superantigen positive patients (%)	Number of samples	Superantigen positive samples (%)
Cardiovascular disorder	4	1 (25)	40	1 (3)
Respiratory disorder	11	3 (27)	47	5 (11)
Abdominal disorder				
Gastrointestinal	18	5 (28)	88	8 (9)
Others	9	4 (44)	105	11 (11)
Neurological disorder	2	0 (0)	7	0 (0)
Trauma	10	1 (10)	50	7 (14)
Burns	10	2 (20)	95	2 (2)
Other disorders	14	0 (0)	42	0 (0)
Total	78	16 (21)	474	34 (7)

[a] Ninety-nine percent cut-off values were used to determine that significant amounts of superantigens were present in the circulation of patients.

Table 2 Characteristics of 16 patients whose plasma superantigen concentrations were elevated above 99% cut-off values.

Patient No.	Sex/Age (yr)	Primary diagnosis	Positive rates[a] (%)	Superantigens detected[b] [Peak concentration (pg/ml)]	Microorganisms isolated (Sites of infection)	Severity of infection			Outcome
						Sepsis	Septic shock	MODS[c]	
1	M/75	Cardiovascular	1/6 (17)	T[27]	*S. aureus* (Sputum)	+	+	+	Alive
2	F/33	Respiratory	2/3 (67)	T[26]	*S. aureus* (Sputum)	+	−	−	Alive
3	M/50	Respiratory	1/1 (100)	A[8], T[35], PA[12]	*K. pneumoniae* (Blood)	+	+	+	Dead
4	M/54	Respiratory	2/12 (17)	PA[30]	*S. aureus* (Blood), *S.pyogenes* (Blood)	+	+	−	Alive
5	M/52	Abdominal (GI)[d]	4/15 (27)	A[16], B[83], C[78], PA[11]	*S. aureus* (Stool, abscess)	+	−	−	Alive
6	M/76	Abdominal (GI)	1/13 (8)	A[10]	*S. aureus* (Sputum)	+	−	−	Alive
7	M/83	Abdominal (GI)	1/3 (33)	T[31]	*Enterococcus* sp., *Enterobacter* sp. (Wound)	+	+	−	Alive
8	M/76	Abdominal (GI)	1/3 (33)	T[27]	*S. aureus* (Wound)	+	+	−	Alive
9	M/73	Abdominal (GI)	1/1(100)	A[9]	Negative	−	−	−	Alive
10	M/50	Abdominal (Others)	1/6 (17)	PA[18]	*S. aureus* (Wound)	+	−	−	Dead
11	M/50	Abdominal (Others)	7/38 (18)	A[11], B[147]	*S. aureus* (Blood)	+	+	+	Alive
12	M/55	Abdominal (Others)	1/3 (33)	B[58]	*K. pneumoniae* (Blood)	+	+	+	Dead
13	M/70	Abdominal (Others)	2/17 (12)	B[153], T[41], PA[182]	*S. aureus* (Blood, ascites)	+	−	−	Alive
14	M/54	Trauma	7/17 (41)	A[15]	*S. aureus* (Abscess)	+	+	−	Alive
15	M/68	Burns	1/57 (2)	A[10]	*S. aureus* (Blood), *P.aeruginosa* (Blood)	+	+	−	Alive
16	M/76	Burns	1/8 (13)	C[103]	*S. aureus* (Wound, sputum)	+	+	−	Dead

[a] Positive rates = No. of superantigen positive samples/No. of total samples.
[b] A: staphylococcal enterotoxin A, B: staphylococcal enterotoxin B, C: staphylococcal enterotoxin C, T: toxic shock syndrome toxin-1, and PA: streptococcal pyrogenic exotoxin A.
[c] MODS: multiple organ dysfunction syndrome.
[d] GI: Gastrointestinal disorders.

Table 3 Relationship between the severity of infection and superantigen detection rates.[a]

Severity of infection	Superantigen positive patients[b]/total patients (%)	Superantigen positive patients[c]/patients with SA or SP[f] infection (%)	Superantigen positive patients[d]/patients without SA or SP[f] infection (%)	Superantigen positive patients[e]/non-survivors (%)
Non-SIRS[g]	1/17 (6)	0/1 (0)	1/16 (6)	0/4 (0)
SIRS without infection	0/21 (0)	0/0 (0)	0/21 (0)	0/1 (0)
Sepsis without shock	5/16 (31)[h]	5/9 (56)	0/7 (0)	0/2 (0)
Septic shock	10/24 (42)[i]	7/15 (47)	3/9 (33)	4/15 (27)
Total	16/78 (21)	12/25 (48)	4/53 (8)	4/22 (18)

[a] Ninety-nine percent cut-off values were used to detect significant concentrations of circulating superantigens.
[b] Superantigen positive patients in total patients.
[c] Superantigen positive patients with SA or SP infection.
[d] Superantigen positive patients without SA nor SP infection.
[e] Superantigen positive patients in non-survivors.
[f] SA: *Staphylococcus aureus*, SP: *Streptococcus pyogenes*.
[g] SIRS: Systemic Inflamatory Response Syndrome.
[h] $p = 0.02$, compared with SIRS without infection.
[i] $p = 0.03$, compared with non-SIRS. $P = 0.003$, compared with SIRS without infection.

superantigen positive samples from individual patients varied from 2 to 67%, except in cases where only one sample was available from each of the patients. These results indicated that a superantigen positive patient did not always exhibit a positive response. As for the species of superantigens detected, SEA was demonstrated in seven patients, SEB in four patients, SEC in two patients, TSST-1 in six patients, and SPEA in five patients (Table 2). In 12 patients, only a single species was detected. Positive responses to multiple species were observed in the following four patients: four species in patient no. 5; three species in patient nos. 3 and 13; and two species in patient no. 11. When biological samples such as blood, aspirate, urine, and/or abscess, were obtained from the superantigen positive patients, *Staphylococcus aureus* was found in the cultures from 12 patients. The *S. aureus*-positive samples included blood samples from four patients. The isolated bacteria, however, were not further examined for their capacity to produce superantigens. The frequency of positive reactions from superantigens was 25% (15/60) in male patients and 6% (1/18) in female patients.

Sepsis was confirmed in 15/16 (94%) of the superantigen positive patients and septic shock in ten patients. Four septic shock patients were complicated by multiple organ dysfunction syndrome. When the 78 patients enrolled in the study were examined, 61 patients (78%) were classified as having systemic inflammatory response syndrome (SIRS), and 17 patients (22%) were non-SIRS (Table 3). Among the 61 SIRS patients, 21 patients were diagnosed as SIRS without infection, 16 patients as sepsis without shock, and 24 patients as septic shock. Superantigen positive rates were 6% in non-SIRS, 0% in SIRS without infection, 31% in sepsis without shock, and 42% in septic shock. Statistical analysis indicated that the superantigen positive rate in septic patients without shock was significantly higher than that in SIRS patients without infection, and that the superantigen positive rate in septic shock patients was significantly higher ($p < 0.05$) than that in non-SIRS patients or SIRS patients without infection.

In the patients whose bacteriological examination revealed *S. aureus* and/or *Streptococcus pyogenes* infection, the superantigen positive rate was 48% (56% in septic patients without shock, 47% in septic shock patients). The positive superantigen detection rate in patients who showed bacteremia with *S. aureus* or *S. pyogenes* was 57% (4/7) and the rate in patients who had non-bacteremic infections with *S. aureus* or *S. pyogenes* was 44% (8/18). In patients where neither *S. aureus* nor *S. pyogenes* infection was confirmed, the superantigen positive rate was 8% (0% in septic patients without shock, 33% in septic shock patients). Death was observed in 22 patients among the patients enrolled in this study. Four of these demonstrated superantigen positive responses and all four had septic shock.

Discussion

In this study, the plasma concentration of five different species of superantigens: SEA, SEB, SEC, TSST-1, and SPEA was examined in an ICU

population, using a sensitive enzyme-linked immunosorbent assay. SEs and SPEs are known to have some homology in their amino acid sequence.[8] TSST-1 differs from SEs and SPEs in amino acid sequence but is similar to toxins of the SE/SPE family in the overall topology.[23,24] The anti-superantigen antibodies used in the current study have previously been confirmed to exhibit no significant cross-reactivity with other related superantigens.[21]

While this study was conducted in a heterogeneous patient population, significant concentrations of circulating superantigens were detected in 34 plasma samples obtained from 16 patients. The plasma concentrations of staphylococcal superantigens, i.e., SEA, SEB, SEC, and TSST-1, were higher than 99% cut-off values in six, four, two, and six patients, respectively. The streptococcal superantigen, SPEA, was positive in three patients. The six patients who had TSST-1 in their circulation did not clinically demonstrate toxic shock syndrome. In this study, plasma samples were collected twice weekly. If a more intensive schedule had been employed, there is a possibility that more superantigen positive patients might have been detected. Although it could not be determined which species of superantigens were more likely to be detected in the blood of ICU patients, it is noteworthy that the superantigen positive rates in septic patients were significantly higher than those in non-septic patients. In the patients whose cultures indicated *S. aureus* or *S. pyogenes* infection, superantigen detection rates were 56% in septic patients without shock and 47% in septic shock patients. On the other hand, one-third of septic shock patients whose cultures demonstrated neither *S. aureus* nor *S. pyogenes* showed superantigenemia.

Furthermore, among the 16 superantigen positive patients, Gram-positive bacteria were not isolated in seven patients. As for the reasons, the limited sensitivity of bacterial culture, inappropriate site selection for bacterial culture, leakage of superantigens into the circulation through the disrupted mucosal barrier that often develops in critically ill patients, and the influence of antibiotics on bacterial cultures may have been associated. While several papers have reported the synergistic lethal effect of superantigens,[25] mortality and the incidence of septic shock or multiple organ dysfunction syndrome were not higher in patients who demonstrated more than two species of superantigens than in those who showed only one species, as shown in Table 2. The actual concentration of superantigens also did not correlate with the severity of infection or outcome. These findings may suggest that the appearance of superantigens in the circulation merely illustrates a phenomenon without any pathologic meanings.

In conclusion, significant levels of superantigens were detected in the plasma of ICU patients. The detection rates of superantigens were especially high in septic patients. Further studies, e.g., an experiment which examines superantigen production activities in microbes isolated from superantigen positive patients, will help to determine the role of superantigens in the pathogenesis of sepsis.

Acknowledgements

We gratefully acknowledge the support of Toray Industries Inc., Pioneering Research Laboratories, Otsu, Shiga, Japan for the measurement of plasma superantigen concentration. We thank K. Miwa, M. Fukuyama, R. Ishii and N. Ida (Toray Industries Inc.) for their excellent technical support and fruitful discussions.

Conflict of interest: No conflict of interest declared.

References

1. Brun-Buisson C, Doyon F, Carlet J, et al. Incidence, risk factors, and outcome of severe sepsis and septic shock in adults. A multicenter prospective study in intensive care units. French ICU Group for Severe Sepsis. *JAMA* 1995;**274**:968—74.
2. Bochud PY, Calandra T, Francioli P. Bacteremia due to viridans streptococci in neutropenic patients: a review. *Am J Med* 1994;**97**:256—64.
3. Rangel-Frausto MS. The epidemiology of bacterial sepsis. *Infect Dis Clin North Am* 1999;**13**:299—312.
4. Sriskandan S, Cohen J. Gram-positive sepsis. Mechanisms and differences from Gram-negative sepsis. *Infect Dis Clin North Am* 1999;**13**:397—412.
5. Opal SM, Cohen J. Clinical Gram-positive sepsis: does it fundamentally differ from Gram-negative bacterial sepsis? *Crit Care Med* 1999;**27**:1608—16.
6. Silverstein R, Norimatsu M, Morrison DC. Fundamental differences during Gram-positive versus Gram-negative sepsis become apparent during bacterial challenge of D-galactosamine-treated mice. *J Endotoxin Res* 1997;**4**:173—81.
7. Becker K, Friedrich AW, Lubritz G, Weilert M, Peters G, Eiff C. Prevalence of genes encoding pyrogenic toxin superantigens and exfoliative toxins among strains of *Staphylococcus aureus* isolated from blood and nasal specimens. *J Clin Microbiol* 2003;**41**:1434—9.
8. Proft T, Moffatt SL, Berkahn CJ, Fraser JD. Identification and characterization of novel superantigens from *Streptococcus pyogenes*. *J Exp Med* 1999;**189**:89—102.
9. Proft T, Webb PD, Handley V, Fraser JD. Two novel superantigens found in both group A and group C Streptococcus. *Infect Immun* 2003;**71**:1361—9.
10. Bergdoll MS. The staphylococcal enterotoxins — an update. In: Jeljaszewicz J, editor. *The Staphylococci*. New York: Gustav Fischer Verlag; 1985.

11. Stevens DL, Tanner MH, Winship J, et al. Severe group A streptococcal infections associated with a toxic shock-like syndrome and scarlet fever toxin A. *New Engl J Med* 1989;**321**:1—7.
12. Bohach GA, Fast DJ, Nelson RD, Schlievert PM. Staphylococcal and streptococcal pyrogenic toxins involved in toxic shock syndrome and related illnesses. *Crit Rev Microbiol* 1990;**17**:251—72.
13. Uchiyama T, Yan XJ, Imanishi K, Yagi J. Bacterial superantigens—mechanism of T cell activation by the superantigens and their role in the pathogenesis of infectious diseases. *Microbiol Immunol* 1994;**38**:245—56.
14. Jupin C, Anderson S, Damais C, Alouf JE, Parant M. Toxic shock syndrome toxin 1 as an inducer of human tumor necrosis factors and gamma interferon. *J Exp Med* 1988;**167**:752—61.
15. Krakauer T. Interleukin-8 production by human monocytic cells in response to staphylococcal exotoxins is direct and independent of interleukin-1 and tumor necrosis factor-alpha. *J Infect Dis* 1998;**178**:573—7.
16. Igarashi H, Fujikawa H, Usami H. Effects of drugs on the pyrogenicity of toxic shock syndrome toxin 1 and its capacity to enhance susceptibility to the lethal effects of endotoxic shock in rabbits. *Rev Infect Dis* 1989;**11**: S210—3.
17. Wells DE, Reeves MW, McKinney RM, et al. Production and characterization of monoclonal antibodies to toxic shock syndrome toxin1 and use of a monoclonal antibody in rapid, one-step enzyme-linked immunosorbent assays for the detection of picogram quantities of toxic shock syndrome toxin 1. *J Clin Microbiol* 1987;**25**:519—21.
18. Miwa K, Fukuyama M, Kunitomo T, Igarashi H. Rapid assay for detection of toxic shock syndrome toxin 1 from human sera. *J Clin Microbiol* 1994;**32**:539—42.
19. Sriskandan S, Moyer D, Cohen J. Detection of circulating bacterial superantigen and lymphotoxin-α in patients with streptococcal toxic-shock syndrome. *Lancet* 1996;**348**:1315—6.
20. Anonymous. American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. *Crit Care Med* 1992;**20**:864—74.
21. Miwa K, Fukuyama M, Sakai R, et al. Sensitive enzyme-linked immunosorbent assays for detection of bacterial superantigens and antibodies against them in human plasma. *Microbiol Immunol* 2000;**44**:519—23.
22. Ryan TA. Significance tests for multiple comparison of proportions, variances, and other statistics. *Psychol Bull* 1960;**57**:318—28.
23. Blomster-Hautamaa DA, Kreiswirth BN, Kornblum JS, Novick RP, Schlievert PM. The nucleotide and partial amino acid sequence of toxic shock syndrome toxin-1. *J Biol Chem* 1986;**261**:15783—6.
24. Acharya KR, Passalacqua EF, Jones EY, et al. Structural basis of superantigen action inferred from crystal structure of toxic-shock syndrome toxin-1. *Nature* 1994;**367**:94—7.
25. Hensler T, Koller M, Geoffroy C, Alouf JE, Konig W. *Staphylococcus aureus* toxic shock syndrome toxin 1 and *Streptococcus pyogenes* erythrogenic toxin A modulate inflammatory mediator release from human neutrophils. *Infect Immun* 1993;**61**:1055—61.