Original Article

Clinical characteristics of patients with severe sepsis and septic shock in relation to bacterial virulence of beta-hemolytic *Streptococcus* and *Streptococcus pneumoniae*

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Aim: Combined detailed analysis of patient characteristics and treatment as well as bacterial virulence factors, which all play a central role in the cause of infections leading to severe illness, has not been reported. We aimed to describe the patient characteristics

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(Charlson comorbidity index [CCI]), treatment (3-h bundle), and outcomes in relation to bacterial virulence of *Streptococcus pneumoniae* and beta-hemolytic *Streptococcus* (BHS).

Methods: This sepsis primary study is part of the larger Focused Outcomes Research in Emergency Care in Acute Respiratory Distress Syndrome, Sepsis and Trauma (FORECAST) study, a multicenter, prospective cohort study. We included patients diagnosed with *S. pneumoniae* and BHS sepsis and examined virulence, defining the high-virulence factor as follows: *S. pneumoniae* serotype 3, 31, 11A, 35F, and 17F; *Streptococcus pyogenes*, *emm 1*; *Streptococcus agalactiae*, III; and *Streptococcus dysgalactiae ssp. equisimilis*, *emm* typing pattern *stG 6792*. Included patients were divided into high and normal categories based on the virulence factor.

Results: Of 1,184 sepsis patients enrolled in the Japanese Association for Acute Medicine's FORECAST study, 62 were included in the current study (29 cases with *S. pneumoniae* sepsis and 33 with BHS). The CCI and completion of a 3-h bundle did not differ between normal and high virulence groups. Risk of 28-day mortality was significantly higher for high-virulence compared to normal-virulence when adjusted for CCI and completion of a 3-h bundle (Cox proportional hazards regression analysis, hazard ratio 3.848; 95% confidence interval, 1.108–13.370; $P = 0.034$).

Conclusion: The risk of 28-day mortality was significantly higher for patients with high-virulence compared to normal-virulence bacteria.

Key words: Beta-hemolytic *Streptococcus*, sepsis, Streptococcus pneumoniae

INTRODUCTION

IN THE PAST two decades, the mortality of severe sepsis has decreased considerably from 35% to less than 20%, likely due to the global acts against sepsis targeting all identified bacteria in the Surviving Sepsis Campaign Guidelines.[1] However, this universal approach is not always sufficient to benefit patients infected with specific pathogens, such as *Streptococcus pneumoniae*,[2] and beta-hemolytic *Streptococcus*,[3] which induce a high mortality rate. To further improve these patients' outcomes, focusing on the specific bacteria responsible for sepsis is a reasonable first step to establish an individualized therapeutic strategy.[4]

Bacterial virulence is "the ability" to enter into a host, survive at host sites, and damage host cells, and plays a central role in the cause of infections, leading to severe sepsis and septic shock.[5] Although prognostic factors of *S. pneumoniae* and beta-hemolytic *Streptococcus* sepsis have been examined in several studies, a combined detailed analysis of patient characteristics, treatment (3-h bundle of sepsis care in the Surviving Sepsis Campaign Guidelines 2016), and bacterial virulence factors has not been reported. Recently, a published study including approximately 50,000 patients concluded that a 3-h bundle of sepsis care was associated with lower risk-adjusted in-hospital mortality. However, in this study, 3-h bundle of sepsis care completion was not significantly associated with in-hospital mortality in Gram-positive bacteremia (odds ratio, 1.01 [confidence interval, 0.98–1.05]).[6] Therefore, it is imperative to examine the baseline characteristics, treatment (3-h bundle), and virulence factors in Gram-positive bacteremia such as *S. pneumoniae* and beta-hemolytic *Streptococcus*.

Thus, the current investigation was the first study to describe the characteristics, treatment (3-h bundle), and outcomes of patients with severe sepsis and septic shock in relation to bacterial virulence of *S. pneumoniae* and beta-hemolytic *Streptococcus*.

METHODS

Study design

THIS IS ONE of the primary investigations of the Focused Outcomes Research in Emergency Care in Acute Respiratory Distress Syndrome, Sepsis and Trauma (FORECAST) study, a multicenter, prospective cohort study of patients infected by *S. pneumoniae* and beta-hemolytic *Streptococcus* with severe sepsis and septic shock. It was undertaken in 59 intensive care units from January 2016 to March 2017 in Japan. Each hospital had a microbiology laboratory. *Streptococcus pneumoniae* and beta-hemolytic *Streptococcus* isolates from sterile clinical samples such as blood, cerebrospinal fluid, pleural effusion, and joint fluid were sent promptly from each clinical laboratory to Keio University School, Department of Infectious Diseases. Laboratory technicians carried out this task in most hospitals; however, emergency physicians were required to do this in some hospitals. The laboratory of Keio University School, Department of Infectious Disease has sufficient experience of current analysis with quality control; the details of the sampling, preservation, and analysis for the examination of virulence factors in individual bacteria were previously reported.[7–10] The results were promptly sent back to the hospitals from the laboratory of Keio University School, Department of Infectious Disease by e-mail from the chief investigator for clinical use.

The study protocol was reviewed and approved by the ethics committee of all institutes in the Japanese Association

for Acute Medicine (JAAM) FORECAST sepsis study groups, Japan. Written informed consent was obtained from each patient or their legally authorized representative based on the decisions made by the local ethics committee, as appropriate. The study was registered with the University Hospital Medical Information Network Clinical Trials Registry (UMIN-CTR ID: UMIN000019702).

Study participants and inclusion criteria

Adult patients (≥16 years) with severe sepsis or septic shock based on sepsis-2 criteria were included in the FORECAST study. We included patients aged 16 years or older who were diagnosed with *S. pneumoniae* and beta-hemolytic *Streptococcus* sepsis. Both *S. pneumoniae* and beta-hemolytic *Streptococcus* were selected, combined, and analyzed as a whole (i.e., representative of *Streptococcus*), because they are similar species and several virulence factors are found across streptococcal species boundaries.[11] Patients with unknown virulence (i.e., detailed examination of virulence factor was not performed because isolates were not sent to the laboratory of Keio University School, Department of Infectious Diseases) were excluded.

Definitions

The Charlson comorbidity index (CCI) is a widely used comorbidity index.[12] Comorbid conditions with a weight of 1 include myocardial infarction, congestive heart failure, peripheral vascular disease, cerebrovascular disease, dementia, chronic pulmonary disease, ulcer disease, mild liver disease, and diabetes mellitus. Diabetes mellitus along with end-organ damage, any tumor, leukemia, and lymphoma have a weight of 2. Moderate or severe liver disease have a weight of 3. Metastatic solid tumors and AIDS have a weight of 6. The total score is calculated by adding the weights.[12]

The presence of systemic inflammatory response syndrome (SIRS), sepsis, severe sepsis, or septic shock was defined according to the definitions of the American College of Chest Physicians/Society of Critical Care Medicine consensus conference statement and its 2003 revision.[13] Illness severity was evaluated with the Sequential Organ Failure Assessment (SOFA) score.[14] The diagnosis of disseminated intravascular coagulation (DIC) was made according to the JAAM DIC diagnostic criteria,[15] with a total score of 4 or more establishing a diagnosis of DIC. Overt DIC was defined[16] and modified[17] according to the International Society of Thrombosis and Hemostasis criteria. Overt DIC was diagnosed when the sum of scores was 5 points or more.

Data sampling

The following data were collected: age, gender, presence of septic shock, location before intensive care unit, primary infection site, co-infection, CCI, vital signs on admission, laboratory data, SIRS score, DIC score, SOFA score, Acute Physiology and Chronic Health Evaluation II (APACHE-II) score, completion and details of a 3-h bundle, 28-day survival, and in-hospital mortality.

High-virulence factor

Bacterial virulence plays a key role in the establishment of bacterial infection; *S. pneumoniae* capsular types and the M protein gene sequence in beta-hemolytic *Streptococcus* are well-known virulence factors.[9,10,18] We defined high-virulence factors in the current study based on the previously reported studies as follows:

Streptococcus pneumonia

Serotype 3, 31, 11A, 35F, and 17F (serotype 3 is a mucoid type, and is associated with serious infections that can lead to fatalities.[18] Because serotype 31, 11A, 35F, and 17F reported higher mortality rates compared to serotype 3, we determined high-virulence factors as serotype 3, 31, 11A, 35F, and 17F in the current study[19]

Beta-hemolytic *Streptococcus*

Streptococcus pyogenes (group A *streptococcus* [GAS]); *emm 1* (*emm 1* has the *sic* gene that inhibits complement-dependent bacteriolysis[10,20]).

Streptococcus agalactiae (group B *streptococcus* [GBS]); III (serotype III is the most common serotype in meningitis. Because the clonal complex 17, sequence type 17 strains are known to be highly virulent, and only included in serotype III, we determined high-virulence factor as serotype III in the current study[8]).

Streptococcus dysgalactiae ssp. *equisimilis* (SDSE); *emm* typing pattern: *stG 6792* (*stG 6792* is the most common typing pattern in invasive SDSE infection[3,9]).

Study end-points

The primary aim of the study was to describe clinical characteristics of patients (patient characteristics, treatment [3-h bundle], and outcome) with severe sepsis and septic shock between high and normal bacterial virulence of *S. pneumoniae* and beta-hemolytic *Streptococcus*.

Statistical analysis

Baseline characteristics were summarized for participants using descriptive statistics. Included patients were divided into two categories based on the virulence factor, high and normal. The distribution of each variable was compared between the two groups defined by the virulence factor (high vs. normal) using the Mann–Whitney *U*-test or Fisher's exact test, depending on variables. Cox proportional hazards regression model of 28-day mortality was used to adjust covariates (CCI, completion of a 3-h bundle, and bacterial virulence factor). All statistical analyses were undertaken using IBM SPSS version 20.0J (IBM, Armonk, NY, USA) and STATA Data Analysis and Statistical Software (version 15.0; StataCorp, College Station, TX, USA). A two-sided *P*-value <0.05 was considered statistically significant.

RESULTS

Demographic factors and patient characteristics, treatment, and outcome of all study patients

OF 1,184 SEPSIS patients enrolled in the JAAM FORECAST study registries, 124 met the inclusion criteria. Of these, 62 patients were excluded due to undetermined virulence. The remaining 62 patients were included in the current study (29 cases with *S. pneumoniae* sepsis and 33 with beta-hemolytic *Streptococcus*). Included patients were divided into two categories based on the virulence factor, high (n = 21) and normal (n = 41, Fig. 1).

The patient age ranged from 33 to 95 years (median, 71 years). Median CCI was 1, and initial SOFA score was 7 (5–11) (median [interquartile range]); 24.1% of patients were infected with bacteria with high virulence (Table 1). In-hospital mortality was 27.1% (n = 16), and 28-day survival was 76.3% (n = 45). Forty-one cases (67.2%) completed a 3-h bundle (Table S1).

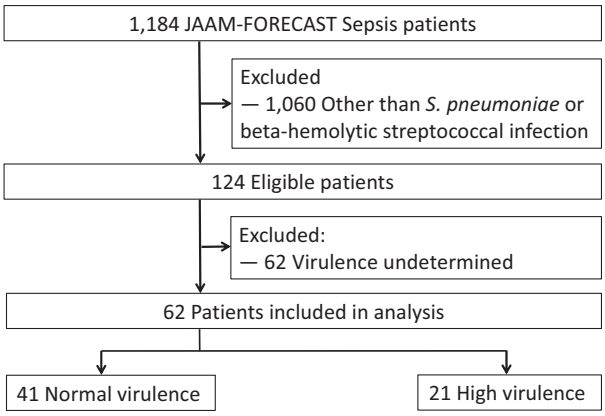

Figure 1. Flowchart of enrolment of study participants. FORECAST, Focused Outcomes Research in Emergency Care in Acute Respiratory Distress Syndrome, Sepsis and Trauma; JAAM, Japanese Association for Acute Medicine.

Comparison of baseline characteristics between normal and high virulence groups

There was no difference in age, gender, rate of septic shock, infection site, or CCI between normal and high virulence factors (Table 2). Illness severity scores such as SIRS, DIC (JAAM), DIC (ISTH), SOFA score, and APACHE-II were not notably different between the high-virulence group and the normal-virulence group.

Comparison of treatment (3-h bundle) between normal and high virulence groups

Completion of a 3-h bundle was not statistically different between the normal-virulence group and the high-virulence group (77.5% versus 52.4%; P = 0.11). Completion rates of each component of the 3-h bundle, such as serum lactate obtainment, broad-spectrum antibiotic treatment, blood cultures obtained before broad-spectrum antibiotic treatment, and 30 mg/kg crystalloid fluid bolus treatment, were relatively higher in the normal-virulence group than in the high-virulence group (Table S2).

Comparison of outcome between virulence factors of normal and high groups

The risk of 28-day mortality was significantly higher for the high-virulence group compared to the normal-virulence group when adjusted for CCI and completion of a 3-h bundle (Cox proportional hazards regression analysis, hazard ratio 3.848; 95% confidence interval, 1.108–13.370; P = 0.034) (Table 3, Fig. S1).

DISCUSSION

IN THE CURRENT study, approximately one-quarter of severe sepsis and septic shock patients with *S. pneumoniae* and beta-hemolytic *Streptococcus* were infected with high-virulence bacteria. Risk of 28-day mortality was significantly higher for high-virulence compared to normal-virulence bacteria when adjusted for patient characteristics (CCI) and treatment (completion of a 3-h bundle).

Hanada *et al*.[21] examined 506 Japanese adults with invasive pneumococcus disease (IPD), and found that host factors (age ≥ 80 years and underlying liver disease) and

Table 1. Baseline characteristics of study patients with severe sepsis and septic shock

Characteristics	
Age (years)	71(65–79)
Gender (male)	44 (71.0)
Septic shock	26 (41.9)
Location before ICU (prior location)	
ER	37 (59.7)
Other	23 (37.1)
ICU	2 (3.2)
Bacteria	
Streptococcus pneumoniae	29 (46.8)
Beta-hemolytic *Streptococcus*	33 (53.3)
Infection site	
Abdomen	1 (1.6)
Lung	23 (37.1)
Skin/STI	22 (35.5)
Infectious endocarditis	2 (3.2)
Others	7 (11.3)
Central nerve system	5 (8.1)
Co-infection	6 (9.7)
Charlson comorbidity index	1 (0–2)
GCS	14 (12–15)
SBP (mmHg)	116 (92–140)
RR (/min)	26 (22–31)
Lactate (mmol/L)	3.1 (2.1–4.8)
Glucose (mg/dL)	130 (104–186)
CRP (mg/dL)	26.9 (14.0–34.4)
BE	−3.9 (−7.1 to 0.5)
Alb (g/dL)	2.8 (2.3–3.2)
SIRS	3 (2–4)
Bilirubin (mg/dL)	0.9 (0.7–1.4)
Creatinine (mg/dL)	1.6 (1.0–2.4)
DIC (JAAM)	4 (2–5)
DIC (ISTH)	3 (1–4)
SOFA score	7 (5–11)
Central nervous system	1 (0–2)
Pulmonary	2 (1–2)
Cardiovascular	1 (0–4)
Hepatic	0 (0–1)
Renal	1 (1–2)
Hematological	1 (0–2)
APACHE-II	22 (17–27)
In-hospital mortality	16 (27.1)

Data are shown as median (interquartile range) or *n* (%).
Missing data: infection site = 11, Glasgow Coma Scale (GCS) = 1, systolic blood pressure (SBP) = 2, respiratory rate (RR) = 1, lactate = 1, glucose = 1, C-reactive protein (CRP) = 1, base excess (BE) = 2, albumin (Alb) = 1, systemic inflammatory response syndrome (SIRS) = 3, bilirubin = 1, creatinine = 1, disseminated intravascular coagulation (DIC) (Japanese Association for Acute Medicine [JAAM]) = 9, DIC (International Society on Thrombosis and Haemostasis [ISTH]) = 12, Sequential Organ Failure Assessment (SOFA) score = 8, central nervous system = 1, pulmonary = 4, cardiovascular = 1, hepatic = 1, renal = 7, Acute Physiology and Chronic Health Evaluation II (APACHE-II) = 16, in-hospital mortality = 3.
ER, emergency room; ICU, intensive care unit; STI, soft tissue infection.

biomarkers (white blood cell count <4,000 cells/μL, serum creatinine ≥2.0 mg/dL, and lactate dehydrogenase ≥300 IU/L) were associated with poor outcomes. In addition, Askim *et al*.[22] undertook a nationwide study regarding IPD in Norway, and concluded that older age and higher severity of disease were mortality risk factors in IPD. However, both studies failed to assess the effects of bacterial virulence and therapeutic intervention, including initial sepsis resuscitation bundle. Regarding beta-hemolytic *Streptococcus* sepsis, Takahashi *et al*.[23] examined clinical aspects in 231 patients with SDSE, 82 with GAS, and 123 with GBS, but treatment factors were not included in the analysis. In the current study, we showed the association between bacterial virulence and patient characteristics, treatment, and outcomes for patients with *S. pneumoniae* and beta-hemolytic *Streptococcus* sepsis.

There are no universal definitions for bacterial virulence;[5] therefore, we defined high-virulence bacteria in the current study based on previous reports.[3,7,8,10] Virulence was particularly determined by *emm* typing in GAS, and serotype in *S. pneumonia* and GBS,[7,8] whereas the rate of *S. pneumonia*, GAS, and GBS between the two subgroups (high-virulence and normal-virulence subgroups) was not different. Although a significant difference was not observed, completion of a 3-h bundle was relatively higher in the normal-virulence group than in the high-virulence group (77.5% versus 53.3%; $P = 0.11$). Thus, we adjusted the covariates such as CCI and completion of a 3-h bundle, and the risk of 28-day mortality was significantly higher for patients with high-virulence compared to normal-virulence bacteria.

Approximately one-third of severe sepsis and septic shock with *S. pneumoniae* and beta-hemolytic *Streptococcus* occurred in patients infected with high-virulence bacteria who, thus, experienced higher mortality. In the era of increasing antibiotic resistance and decreasing antibiotic drug development, it is crucial that that targeting virulence becomes an attractive therapeutic strategy, as Webb and Kahler proposed.[5] Our study could contribute to the development of

Table 2. Comparison of characteristics between virulence factors (normal vs. high) in patients with severe sepsis and septic shock

Characteristics	Normal virulence (*N* = 41)	High virulence (*N* = 21)	*P*-value
Age, (years)	71 (65–78)	75 (63–81)	0.566
Gender (male)	25 (61.0%)	18 (69.4%)	0.079
Septic shock[†]	14 (34.2%)	12 (57.1%)	0.110
Location before ICU (prior location)			0.858
ER	27 (62.5%)	12 (57.1%)	
Other	14 (35.0%)	8 (38.1%)	
ICU	1 (2.5%)	1 (4.8%)	
Bacteria			0.423
Streptococcus pneumoniae	21 (51.2%)	8 (38.1%)	
Beta-hemolytic *Streptococcus*	20 (48.8%)	13 (61.9%)	
Infection site			0.064
Abdomen	1 (2.4%)	0 (0%)	
Lung	15 (36.6%)	8 (38.1%)	
Skin/STI	14 (34.2%)	9 (42.9%)	
Infectious endocarditis	1 (2.4%)	1 (4.8%)	
Others	4 (9.8%)	2 (9.5%)	
Central nervous system	5 (12.2%)	0 (0%)	
Co-infection	6 (14.6%)	0 (0%)	0.321
Charlson comorbidity index	1 (0–2)	1 (0–2)	0.811
GCS	14 (10–15)	15 (13–15)	0.283
SBP (mmHg)	118 (94–143)	109 (91–139)	0.467
RR (/min)	25 (22–30)	29 (21–38)	0.548
Lactate (mmol/L)	3.1 (2.1–4.9)	3.8 (2.3–5.7)	0.919
Glucose (mg/dL)	149 (111–202)	122 (99–137)	0.048
CRP (mg/dL)	25.9 (12.3–33.2)	29.8 (17.1–39.3)	0.104
BE	−3.5 (−5.7 to 0.4)	−5.8 (−10.1 to −3.3)	0.051
Alb (g/dL)	2.9 (2.3–3.4)	2.6 (2.3–3.0)	0.488
SIRS	3 (2–4)	3 (3–4)	0.407
Bilirubin	0.8 (0.7–1.2)	1.3 (0.7–2.1)	0.113
Creatinine	1.4 (1.0–2.2)	1.7 (1.5–2.6)	0.128
DIC (JAAM)	4 (2–6)	4 (3–5)	0.861
DIC (ISTH)	3 (1–5)	3 (1-4)	0.574
SOFA score	7 (5–10)	9 (7–13)	0.081
Central nervous system	1 (0–2)	0 (0–2)	0.283
Pulmonary	2 (1–2)	2 (1–2)	1.000
Cardiovascular	1 (0–4)	4 (0–4)	0.204
Hepatic	0 (0–1)	1 (0–2)	0.065
Renal	1 (0–2)	2 (1–3)	0.101
Hematological	1 (0–2)	1 (1–2)	0.451
APACHE-II	22 (17–29)	21 (17–26)	0.553

Missing data: normal virulence, infection site = 9, Glasgow Coma Scale (GCS) = 1, systolic blood pressure (SBP) = 1, respiratory rate (RR) = 1, lactate = 1, glucose = 1, C-reactive protein (CRP) = 1, base excess (BE) = 2, albumin (Alb) = 1, systemic inflammatory response syndrome (SIRS) = 3, bilirubin = 1, creatinine = 1, disseminated intravascular coagulation (DIC) (Japanese Association for Acute Medicine [JAAM]) = 9, DIC (International Society on Thrombosis and Haemostasis [ISTH]) = 9, Sequential Organ Failure Assessment (SOFA) score = 5, central nervous system = 1, pulmonary = 2, cardiovascular = 1, hepatic = 1, renal = 5, Acute Physiology and Chronic Health Evaluation II (APACHE-II) = 12; high virulence, infection site = 2, SBP = 1, lactate = 1, DIC (ISTH) = 3, SOFA score = 3, pulmonary = 2, renal = 2, (APACHE-II) = 4.
ER, emergency room; ICU, intensive care unit; STI, soft tissue infection.
[†]Septic shock or lactate >4 mmol/L.

Table 3. Prognostic factors of 28-day mortality in patients with severe sepsis and septic shock, Cox's proportional hazards regression analysis

	HR	95% CI	*P*-value
Virulence (high versus normal)	3.849	1.108–13.370	0.034
Charlson comorbidity index	1.083	0.730–1.609	0.689
Completion of a 3-h bundle	1.277	0.368–4.436	0.700

CI, confirmation interval; HR, hazard ratio.

antibody treatments against bacterial virulence in *S. pneumoniae* and beta-hemolytic *Streptococcus* sepsis.

This study has several limitations. First, due to the small number of patients included in the analysis, *S. pneumoniae* and beta-hemolytic *Streptococcus* could not be examined independently. This might have caused the heterogeneity in the study populations. Thus, further study with larger populations with each pathogen should be undertaken to examine the hypothesis in the current study. However, *S. pneumoniae* and beta-hemolytic *Streptococcus* were analyzed as one category because several virulence factors in those bacteria are found across streptococcal species boundaries.[11] Second, a detailed examination of virulence factors was not carried out in half of the cases; therefore, selection bias would occur. Emergency physicians, not laboratory technicians, were required to send clinical samples to Keio University in some hospitals. This seems to be the main reason for low numbers of cases submitted. Prospective studies with detailed examination of virulence factors for all eligible cases are required. Third, facility factors were not examined due to the unavailability of the dataset in the current study. This might have greatly affected the outcome. Finally, pneumococcal vaccination status was not obtained.

CONCLUSIONS

THE RISK OF 28-day mortality was significantly higher in patients with severe sepsis and septic shock infected with high-virulence compared to normal-virulence bacteria when adjusted for patient characteristics (CCI) and treatment (completion of a 3-h bundle).

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DISCLOSURE

Approval of the research protocol: The study protocol was reviewed and approved by the ethics committee of all participating institutes in the JAAM study group, Japan (IRB No. 015-0021 on Hokkaido University, the representative for FORECAST).
Informed consent: N/A.
Registry and the registration no. of the study/trial: The University Hospital Medical Information Network Clinical Trials Registry (UMIN-CTR ID: UMIN000019702), Date of registration: 11/09/2015, https://upload.umin.ac.jp/cgi-open-bin/ctr_e/ctr_view.cgi?recptno=R000022760
Animal studies: N/A.
Conflict of interest: Dr. Seitaro Fujishima reports personal fees from Asahi Kasei Japan and Takeda Pharmaceutical, and grants from Chugai Pharmaceuticals, Daiichi-Sankyo, Otsuka Pharmaceutical, Pfizer, Astellas Pharma, Shionogi, and Teijin Pharma, outside the submitted work. Dr. Satoshi Gando reports personal fees from Asahi Kasei Japan and Asahi Kasei America. The other authors have no conflict of interest.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Details of treatment (3-h bundle) of study patients.

Table S2. Comparison of treatment (3-h bundle) between virulence factors (normal versus high).

Figure S1. Cox proportional hazards regression.