Clinical characteristics, risk factors, immune status and prognosis of secondary infection of sepsis: a retrospective observational study

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Abstract

Background: Secondary infection has a higher incidence in septic patients and affects clinical outcomes. This study aims to investigate the clinical characteristics, risk factors, immune status and prognosis of secondary infection of sepsis.

Methods: A four-year retrospective study was carried out in Zhongshan Hospital, Fudan University, enrolling septic patients admitted between January, 2014 and January, 2018. Clinical data were acquired from medical records. CD14+ monocyte human leukocyte antigen-D related (HLA-DR) expression and serum cytokines levels were measured by flow cytometry and enzyme-linked immunosorbent assay (ELISA) respectively.

Results: A total of 297 septic patients were enrolled, 92 of whom developed 150 cases of secondary infections. Respiratory tract was the most common site of secondary infection (n = 84, 56%) and Acinetobacter baumanii the most commonly isolated pathogen (n = 40, 31%). Urinary and deep venous catheterization increased the risk of secondary infection. Lower HLA-DR expression and elevated IL-10 level were found in secondary infection group. The expected prolonged in-hospital stay owing to secondary infection was 4.63 ± 1.87 days. Secondary infection was also associated with higher in-hospital, 30-day and 90-day mortality. Kaplan-Meier survival analysis and Log-rank test revealed that secondary infection group had worse survival between day 15 and day 90.

Conclusions: Urinary and deep venous catheter indwelling increased the risk of developing secondary infection, in which underlying immunosuppression also played a role. Secondary infection affected outcomes of septic patients and prolonged in-hospital length of stay.

Background

Sepsis accounts for a considerable number of hospital and intensive care unit (ICU) admission and adds to the overall in-hospital mortality [1,2]. Lack of consensus and
knowledge in its pathological mechanism has posed a threat to patient management. After proper treatment, conditions of many septic patients became stable. However, some other patients developed secondary infection which led to the aggravation of disease and multiple organ dysfunction syndrome (MODS).

Previous studies have provided some findings on the risk factors of developing secondary infection, such as age, severity of disease on admission, length of stay (LOS) in ICU and invasive procedures [3,4]. Some studies also focused on the association between secondary infection and the prognosis of septic patients but the results were inconsistent in how secondary infection influenced the prognosis and whether it was the major cause of death [5,6].

It has also been widely studied that the underlying immune dysfunction of sepsis could lead to secondary infection. The early phase of sepsis features hyper inflammation caused by the systemic release of pro-inflammatory cytokines called “cytokine storm” [7,8]. Immunosuppression is then observed at later phase of sepsis as a result of the imbalance of pro- and anti-inflammatory activities [9]. Sepsis could lead to a variety of mechanisms such as the apoptosis and autophagy of immune cells, endotoxin tolerance and relevant center nervous system regulation, which is presented as immunosuppression consequently [8,10,11]. CD14+ monocyte human leukocyte antigen-D related (HLA-DR) expression is an effective biomarker of immune status, which reflects net sum of pro- and anti-inflammatory process during sepsis [12-14]. Low HLA-DR expression is associated with immunosuppression and higher risk of secondary infection, especially during early phase of sepsis [15-19]. Serum cytokine levels are also commonly used by clinicians to monitor immune status. A higher release of anti-inflammatory cytokines such as IL-10, together with acute pro-inflammatory activities were found in the patients prone to secondary infection [20-24].
Because of the illuminating but inconsistent findings of previous studies, the clinical characteristics, risk factors and the prognosis of secondary infection of sepsis were further investigated. Additionally, the association between immune status and secondary infection of sepsis based on data of HLA-DR expression and serum cytokines levels were also explored in the current study.

Materials And Methods

Study setting and population

A retrospective study was carried out in emergency intensive care unit (EICU) of Zhongshan Hospital, Fudan University, Shanghai, China. Patients diagnosed with sepsis on admission between January, 2014 and January, 2018 were enrolled in this study. The diagnosis of sepsis referred to The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3), namely suspected infection with Sequential Organ Failure Assessment (SOFA) score ≥ 2 [2]. Information of infection and SOFA score were acquired form Electronic Medical Record System (EMRS). Patients were excluded if they had one of the following conditions: ① under the age of 18; ② suffering chronic heart failure (New York heart function assessment - IV), advanced malignancy, end-stage liver (Child-Pugh C) or kidney diseases (CKD-5); ③ having received in-hospital treatment in other hospitals prior to being admitted to this hospital; ④ in-hospital LOS less than 48 hours. Anti-infection treatments of all included patients were applied by experienced physicians based on either etiological evidence or empirical therapy plan. The study was approved by the Ethics Committee Study Board of Zhongshan Hospital, Fudan University (record number: 2006-23).

Diagnosis of secondary infection

Secondary infection was diagnosed according to CDC/NHSN Surveillance Definition Of
Health Care-Associated Infection And Criteria For Specific Types Of Infections In The Acute Care Setting [25]. Clinical information used to identify secondary infection such as signs/symptoms and results of laboratory tests such as pathogen cultures were acquired from EMRS. Only the newly-onset nosocomial infections identified later than 48 hours after admission were classified as secondary infections. The time of the onset of secondary infection was the day when positive cultures were collected, or when signs/symptoms emerged if no positive culture was gained. Infections identified after leaving hospital were not documented. An infection caused by multiple pathogens but identified at the same time and same site was considered as one infection. Three experienced researchers were responsible for the diagnosis of secondary infection.

Data collection

EMRS and Computerized Physician Order Entry (CPOE) were screened for available data. The following data of each patient were collected: ① baseline characteristics: age, gender, comorbidity and smoking history; ② site of primary infection; ③ index of severity of the disease at the time of admission: Acute Physiology and Chronic Health Evaluation II (APACHE II) score, SOFA score and hemodynamic status; ④ interventions such as the use of glucocorticoids, anticoagulation therapy, mechanical ventilation, urinary catheterization, deep venous catheterization, continuous renal replacement therapy and blood transfusion (whether those interventions were applied before or after the onset of secondary infection was noticed); ⑤ occurrence time, site and pathogen of secondary infection; ⑥ LOS in hospital and ICU, the outcome of hospital stay.

Measurement of monocyte HLA-DR expression and serum levels of cytokines

In order to explore the underlying immune mechanism of secondary infection, we acquired
the data from Database of Clinical Sample and Information for Sepsis of Zhongshan Hospital, an database founded in 2008 intended for the collection and perseveration of clinical samples of sepsis. According to the guideline of database, the peripheral blood samples were collected in the BD Vacutainer® tubes at day 1, 3 and 7 after admission. In some patients, sample at day 3 and 7 were not collected. As the nature of an retrospective study, data of only a part of the included patients were available. To explore CD14⁺ HLA-DR⁺ monocytes expression, a following double color staining was utilized: a fluorescein conjugated (FITC)-CD14, allophycocyanin conjugated (APC)-HLA-DR (BD pharangen, CA, USA), according to manufacturer’s instructions. Appropriate isotype controls were run with healthy controls and used for compensation and gating blood samples. Subsequently, samples were analyzed on a 18-parameter BD LSRFortessa machine with FlowJo software (Tree Star Inc, OR, USA). For monocytes, debris was excluded and 10000 CD14⁺ events were collected per sample. HLA-DR expression was expressed as the percentage of CD14⁺ HLA-DR⁺ monocyte among all CD14⁺ monocyte.

The level of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), IL-8 and IL-10 were measured by ELISA method (R&D System, MN, USA) according to manufacturer’s instructions. The experiment of flow cytometry and ELISA were conducted right after the samples were collected and the results were recorded in the database. In this retrospective study, the results were directly acquired from the database.

**Statistical analysis**

The Kolmogorov-Smirnov test was used to verify the normality of all data. Normally distributed data were expressed as means and standard deviation (SD). Abnormally distributed continuous data were expressed as median and the 25th and 75th quartiles.
Categorical data were expressed as frequency and percentage.

The risk factors of secondary infection of septic patients were explored by a two-step method. Firstly, univariate analysis were conducted. Covariates included age, gender, comorbidities, smoking history, site of primary infection, hemodynamic status and severity of disease on admission, HLA-DR expression and serum cytokines levels and clinical interventions before onset of secondary infection. Student’s t test was used to compare normally distributed data and Mann-Whitney U test was utilized to compare abnormally distributed data. Categorical data were compared by Pearson’s chi-square test or Fisher’s exact test when appropriate. Secondly, covariates with statistical significance in univariate analysis were tested in multivariate binary logistic regression analysis to identify the independent risk factors by means of Backward: Conditional method. Because the data missing of HLA-DR expression and serum cytokine levels, they were not brought into multivariate analysis.

In our study, we treated in-hospital LOS as an outcome of secondary infection, rather than a potential risk factor. A multistate model with 4 states (state 0: admission, state 1: development of secondary infection, state 2: being discharged alive, state 3: in-hospital death) was performed using “etm” package in R in order to explore the influence of secondary infection on in-hospital LOS, where the data of patients with an in-hospital LOS longer than 100 days were omitted to eliminate the impact of extreme cases (see Additional file 1: Figure S1) [3]. Survival analysis was conducted by Kaplan-Meier method and Log-rank test was used to compare survival curves and it was conducted in every division once two curves had intersections. The two-step method was also used to explore the risk factors of mortality. Univariate analysis was conducted first and followed by multivariate binary logistic regression analysis. Secondary infection was among covariates, together with age, gender, comorbidities, smoking history, site of primary
infection, hemodynamic status and severity of disease on admission, clinical interventions and ICU and in-hospital LOS.

All statistical analyses were two-sided, and the significance level was set to $P < 0.05$. We checked the model assumptions before using each statistical methods. Statistical analysis was conducted on SPSS 25.0 (SPSS Inc., IL, USA) and R 3.5.1 (R Development Core Team).

Results

**Characteristics of septic patients**

From January, 2014 to January, 2018, a total of 297 patients were enrolled in this study. A flowchart to illustrate the recruitment of study samples was shown in Figure 1. 195 were men and the median age was 66 years. 241 patients had comorbidities (82.1%). Respiratory tract was the most common site of primary infection ($n = 216, 72.7\%$). Other sites of infection included abdomen ($n = 62, 20.9\%$), urinary tract ($n = 22, 7.4\%$), skin and soft tissue ($n = 12, 4\%$) and blood stream ($n = 4, 1.3\%$), 21 patients had more than one infection sites ($7.1\%$). 77 patients had septic shock on admission (25.9%). The baseline characteristics of the enrolled patients were shown in Table 1.

**Characteristics of septic patients with secondary infection**

150 cases of secondary infection were developed in 92 patients, 26 of whom had multiple secondary infections. Respiratory tract was the most common site of secondary infection ($n = 84, 56\%$), followed by urinary tract ($n = 42, 28\%$), blood stream and disseminated infection ($n = 18, 12\%$), abdomen ($n = 5, 3.3\%$) and skin and soft tissue ($n = 1, 0.7\%$). Day 8 was the median time of developing the first secondary infection. *Acinetobacter baumanii* ($n = 40, 26.7\%$), *Klebsiella pneumoniae* ($n = 21, 14\%$), *Enterococcus faecium* ($n = 11, 7.3\%$), *Candida tropicalis* ($n = 9, 6\%$), *Pseudomonas aeruginosa* ($n = 9, 6\%$) and *Staphylococcus aureus* ($n = 9, 6\%$) were common identified pathogens. In 23 cases,
pathogens were not identified. The characteristics of secondary infections were shown in Table 2 and distribution of pathogens, time of onset and diagnostic criteria of each infection were shown in Table S1 (see Additional file 2).

**Risk factors of secondary infection in septic patients**

No statistical significance existed between septic patients with and without secondary infection concerning age, gender, comorbidity and site of primary infection. In univariate analysis, statistical significance was found in severity of illness on admission (APACHE II score: \( P < 0.001 \); SOFA score: \( P = 0.007 \)) and some interventions before the onset of secondary infection such as the use of mechanical ventilation (OR 2.752, 95% CI 1.604 to 4.721, \( P < 0.001 \)), urinary catheterization (OR 5.292, 95% CI 2.997 to 9.343, \( P < 0.001 \)), deep venous catheterization (OR 4.494, 95% CI 2.629 to 7.680, \( P < 0.001 \)) and blood transfusion (OR 2.152, 95% CI 1.18 to 3.925, \( P = 0.011 \)) (Table 1). Factors with statistical significance were tested under multivariate logistic regression analysis and urinary catheterization (OR 3.384, 95% CI 1.791 to 6.392, \( P < 0.001 \)) and deep venous catheterization (OR 2.608, 95% CI 1.422 to 4.784, \( P = 0.002 \)) remained statistical significant (Table 3).

**The association between HLA-DR expression, cytokine levels and secondary infection of sepsis**

Data of a part of patients were available for HLA-DR expression and cytokines. The exact numbers were shown in Table 1. In the univariate analysis of the risk factors of secondary infection, statistical significance was found in HLA-DR expression at day 3 (\( P = 0.048 \)), IL-6 level at day 1 (\( P = 0.025 \)), IL-8 level at day 3 (\( P < 0.001 \)) and IL-10 level at day 7 (\( P = 0.035 \)). The results were shown in Table 1, Figure 2 and Additional file 3: Table S2.
Although statistical significance was not found at every time point, a trend of decrease of HLA-DR expression and increase of IL-10 level in secondary infection group was observed, which is indicative of immunosuppression (Figure 2A & B). Interestingly, a reverse trend of dynamic change was found between two pro-inflammatory cytokines IL-6 and IL-8 in both secondary infection and non-secondary infection groups (Figure 2C & D).

Representative flow cytometry profiles for HLA-DR expression were shown in Figure 3.

**The association between secondary infection and the outcomes of sepsis**

Secondary infection group had longer LOS in hospital and ICU than non-secondary infection group (in-hospital LOS: $P < 0.001$; ICU LOS: $P < 0.001$) (Table 1). Multistate model revealed expected prolonged LOS in hospital was 4.63 days (Figure 4). In-hospital, 30-day, 90-day mortality was 45.7%, 34.8%, 42.4% in secondary infection group and 25.4%, 23.4% and 25.4% in non-secondary infection group respectively (OR 2.472, 95% CI 1.474 to 4.145, $P = 0.001$; OR 1.744, 95% CI 1.019 to 2.985, $P = 0.041$; OR 2.165, 95% CI 1.288 to 3.640, $P = 0.003$, respectively). The proportion of developing secondary infection were 44.7% and 24.6% in in-hospital mortality group and survival group respectively (OR 2.472, 95% CI 1.474 to 4.145, $P = 0.001$) (see Additional file 4: Table S3). Multivariate binary logistic regression analysis also found out that secondary infection was an independent risk factor of in-hospital mortality (OR 3.476, 95% CI 1.599 to 8.219, $P = 0.003$) (see Additional file 5: Table S4). Kaplan-Meier survival curves and Log-rank test revealed no difference between two groups before day 15 ($P = 0.426$) (see Additional file 6: Figure S2). But non-secondary infection group had a better survival between day 15 and day 90 ($P < 0.001$) (Figure 5) and subgroup analysis showed that the difference remained significant in patients with and without septic shock ($P = 0.04$ and $P < 0.001$) (see Additional file 7: Figure S3).
Discussion

Our study confirmed a high incidence of secondary infection in septic patients (31.0%). Moreover, urinary and deep venous catheterization could bring higher risk in developing secondary infection. Patients with secondary infection had higher chances of immunosuppression. Secondary infection also affected the prognosis, which featured poor survival at later period (> 15 days after admission). Expected prolonged in-hospital LOS was 4.63 days in secondary infection group.

A recent meta-analysis revealed that lower respiratory tract infection was the most common nosocomial infection in general hospital and *Pseudomonas aeruginosa*, *Escherichia coli*, *Acinetobacter baumannii* and *Klebsiella pneumoniae* were among most common pathogens [26]. Similar findings were discovered in our study. The high pathogenicity of such Gram-negative bacteria was due to drug resistant and invasive procedures which serves as approach of the invasion of pathogens [27]. Increased susceptibility of secondary infection could be pathogen-specific due to the different patterns of immune barrier destruction. Opportunistic bacterial and fungal infections were more prevalent as a consequence of immunosuppression [28-30]. There was also higher chance of viral reactivation and co-infection in the case of immunosuppression [30]. In our study, 6 cases of secondary infection were defined as disseminated infection, which were likely caused by viruses according to the CDC criteria [25,30]. However, it’s possible that some pathogenic microorganisms, especially viruses, were not identified due to limited testing technologies.

We found higher APACHE II and SOFA scores on admission in patients with secondary infection, which were similar to previous studies [3,4,31]. Although illness severity was not found to be an independent risk factor of secondary infection. It could be explained that the more severely ill patients died mostly at the very early period of disease before
developing secondary infections, which might impact the true association between the risk and illness severity.

It’s widely acknowledged that catheter indwelling was a major cause of nosocomial infection [32-34]. We found that urinary catheterization was an independent risk factor of secondary infection. A study revealed that catheter-associated urinary tract infection was not only affected by duration of urinary catheterization, but also the presence of another site of nosocomial infection, which was confirmed by our study that many cases of secondary infections in urinary tract were subsequent to secondary infections at other sites [35]. Deep venous catheterization was also common in ICU setting. Our finding was consistent with the study by van Vught et al. that it was also an independent risk factor of secondary infection [4]. Also, mechanical ventilation and blood transfusion were found to be risk factors in univariate analysis. The need for mechanical ventilation of critical ill patients incurred high prevalence of ventilator-associated pneumonia, which accounted for nearly half of nosocomial infections and was found to be an independent risk factor of secondary infection in some studies [3,4,36]. But the result of multivariate analysis of our study was not consistent with previous findings. As we did not tell apart invasive and non-invasive ventilation due to limited medical record and the duration of ventilation was not recorded, the result should be interpreted cautiously. The association between blood transfusion and secondary infection of sepsis could be induced by transfusion-related immune modulation (TRIM) caused by the altered immune function [37-40]. Immune status of septic patients and its underlying mechanism have been widely studied. Innate immune function was altered as a dysfunction of neutrophils, monocytes, dendritic cells and myeloid-derived suppressor cells (MDSCs) which causes altered first-line of defense, inhibition of T cell proliferation, altered inflammatory response and incomplete activation of T cells [8]. Adaptive immune function was also altered as sepsis affects the
effector functions and phenotypes of T cells, B cells and innate-type lymphocytes [8]. HLA-DR and cytokines were chosen to reflect the immune status in this study. HLA-DR was a marker reflecting both innate and adaptive immune function and lower expression indicates immunosuppression [8]. IL-10 was an anti-inflammatory cytokine and elevated level reflected the down-regulation of inflammation process. It might generate MDSCs and enhances the immunosuppression during sepsis [20,41]. In secondary infection group of this study, HLA-DR expression was lower and IL-10 level showed a trend of increase, which was a sign of immunosuppression. A more severe pro-inflammatory response in secondary infection group presented as a higher IL-6 and IL-8 level, was also observed, which were both pro-inflammatory cytokines. This confirmed that higher pro- and anti-inflammatory process might exist at the same time in septic patients with secondary infection [21,23]. A reverse trend of dynamic change between IL-6 and IL-8 was noticed, though they were both pro-inflammatory cytokines. This might be explained by that the increase of IL-6 demonstrated the progress of inflammation, as the blood sample collected at day 3 and 7 were more often from severely ill patients. IL-8, as we hypothesized, might be involved in early phase inflammatory process rather than later phase and thus showed a trend of decrease. As the dynamic changes were only statistical significant in some timepoints, studies with a larger sample size are needed to further the study. Those results enlightened us that the identification and risk stratification of immunosuppression and the therapies that boost immunity could be beneficial to the prevention of secondary infection [13,42,43].

Secondary infection was observed to prolong the time of hospitalization under a multistate model, which was an result of the complexity of disease requiring longer in-hospital treatment. Longer LOS in turn increased the chance in developing secondary infection. When it comes to mortality, secondary infection was an independent risk factor of in-
hospital death in this study. Survival analysis demonstrated that patients with secondary infection had worse outcome after first 15 days. In the first 15 days, secondary infection group even had better survival. That could be explained by that patients who were severely sick died earlier before they developed secondary infections. This was consistent with the previous concept that the mortality of patients who survived that early period was more likely affected by secondary infection [13]. A re-increased microbiological burden revealed by positive blood cultures at later phase of sepsis (> 15 days) was observed in the study by Otto et al., which was indicative of secondary infection and poor outcomes [44]. However, Goldenberg et al. addressed that secondary infection was not the main cause of death in sepsis as they found only a small portion (14%) of septic patients died with an evidence of secondary infection. Some studies found that mitochondrial dysfunction, microvascular leak or even activity of daily living could serve as causes of death from sepsis [5,28].

This study had some limitations. First, the sample size was relatively small as a single-center study. Second, some clinical data such as the use of antibiotics, the exact dose of glucocorticoids, duration of mechanical ventilation and catheter indwelling were not documented due to the limited medical record, which blocked us from exploring the dose-response relationship. Third, data of HLA-DR expression and serum cytokine levels of many patients were not available as a retrospective study. Thus the association between immunosuppression and secondary infection was worthy of further prospective research with a larger sample size.

Conclusion

Invasive operations such as urinary catheterization and deep venous catheterization increased the risk in developing secondary infection, in which underlying immunosuppression also played a role. Secondary infection influenced outcomes of
patients as it prolonged expected in-hospital LOS and increased mortality in patients who survived early period of sepsis. The monitoring of immune status and proper care to minimize the invasion of pathogens were keys to lower incidence of secondary infection.

Abbreviations

APACHE II: Acute Physiology and Chronic Health Evaluation II
APC: Allophycocyanin
CDC: Centers for Disease Control and Prevention
CI: Confidential interval
CKD: Chronic kidney disease
CPOE: Computerized Physician Order Entry
ELISA: Enzyme-linked immunosorbent assay
EMRS: Electronic Medical Record System
FITC: Fluorescein-5-isothiocyanate
HLA-DR: Human leukocyte antigen-D related
ICU: Intensive care unit
IL: Interleukin
LOS: Length of stay
MDSC: Myeloid-derived suppressor cells
MODS: Multiple organ dysfunction syndrome
NHSH: National Healthcare Safety Network
SD: Standard deviation
SOFA: Sequential Organ Failure Assessment
TNF-α: Tumor necrosis factor-α
TRIM: Transfusion-related immune modulation
Declarations

**Ethics approval and consent to participate**

The clinical study protocol was approved by the Ethics Committee Study Board of Zhongshan Hospital, Fudan University, Shanghai, China (record number 2006-23). Written informed consent was obtained from subjects or their legal surrogates before enrollment.

**Consent for publication**

Not applicable.

**Availability of data and material**

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Competing interests**

The authors declare that there are no conflicts of interest.

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**Authors’ contributions**

ZS, CT, SD, YC, YH and JZ contributed to the study design. YC, YH, JZ, YS, JH, JY, PW, YF and SL contributed to the data collection, statistical analysis and the interpretation of the results. JH, YY and LY performed the experimental analysis of measurement of monocyte HLA-DR expression and serum cytokines levels. YC, YH, JZ, YS, JW, YY, KL, ZS, CT and SD contributed to the drafting and revision of the manuscript. All authors have approved the final draft of the manuscript.

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Tables
Table 1. Characteristics of septic patients classified according to developing secondary infection or not

| Baseline characteristics                  | With secondary infection n = 92 | Without secondary infection n = 205 | P value |
|-------------------------------------------|--------------------------------|---------------------------------|---------|
| Age, median (25th,75th)                   | 66.5 (53.5-78.8)                | 65 (52.3-75)                    |         |
| > 65 years, n (%)                         | 50 (54.3)                      | 105 (51.2)                     |         |
| Men, n (%)                                | 63 (68.5)                      | 132 (64.4)                     |         |
| Comorbidities, n (%)                      |                                |                                |         |
| None                                      | 16 (17.4)                      | 40 (19.5)                      |         |
| Hypertension                              | 42 (45.7)                      | 82 (40)                        |         |
| Other cardiovascular disease a            | 15 (16.3)                      | 25 (12.2)                      |         |
| Diabetes mellitus                         | 23 (25)                        | 46 (22.4)                      |         |
| Cerebrovascular disease                   | 6 (6.5)                        | 13 (6.3)                       |         |
| Respiratory disease                       | 9 (9.8)                        | 23 (11.2)                      |         |
| Hepatitis and cirrhosis                   | 3 (3.3)                        | 10 (4.9)                       |         |
| Renal insufficiency                       | 4 (4.3)                        | 15 (7.3)                       |         |
| Malignancy                                | 8 (8.7)                        | 17 (8.3)                       |         |
| Immunosuppression                         | 12 (13)                        | 24 (11.7)                      |         |
| Smoker, n (%)                             | 32 (34.8)                      | 69 (33.7)                      |         |
| Site of infection, n (%)                  |                                |                                |         |
| Respiratory tract                         | 70 (76.1)                      | 146 (71.2)                     |         |
| Abdomen                                   | 15 (16.3)                      | 47 (22.9)                      |         |
| Urinary tract                             | 8 (8.7)                        | 14 (6.8)                       |         |
| Skin and soft tissue                      | 6 (6.5)                        | 6 (2.9)                        |         |
| Blood stream                              | 2 (2.2)                        | 2 (1)                          |         |
| More than one sites       | 8 (8.7) | 13 (6.3) |
|----------------------------|---------|----------|
| In shock on admission, n (%)| 28 (30.4) | 49 (22.4) |
| Severity of disease, median (25th,75th) | | |
| APACHE II score          | 17 (9.25-22) | 11 (7-18) |
| SOFA score               | 4 (3-8) | 4 (2.5-6) |
| Monocyte HLA-DR expression (%) | | |
| Day 1, mean (SD)         | 31.6 (14.3) | 34.5 (14.9) |
| Day 3, median (25th,75th) | 28.6 (18.8-42) | 41.1 (27.5-50.4) |
| Day 7, median (25th,75th) | 29.6 (14.3-35.1) | 33.2 (13.8-65.4) |
| Level of serum cytokines (pg/ml) | | |
| Day 1, median (25th,75th) | | |
| IL-6                      | 26.8 (13.6-363.5) | 21.1 (7.5-58.2) |
| IL-8                      | 36.3 (18.7-70) | 22.9 (12-77.5) |
| IL-10                     | 8.6 (5.4-24) | 10 (9.3-17.8) |
| Day 3, median (25th,75th) | | |
| IL-6                      | 628.5 (23.5-1694.5) | 640.5 (17.8-942.3) |
| IL-8                      | 29.89 (20-106) | 8 (4.9-15.6) |
| IL-10                     | 21.7 (6.4-35.3) | 14.6 (5-27.7) |
| Day 7                     | | |
| IL-6, median (25th,75th)  | 921 (652-1377) | 754 (584-1004) |
| IL-8, median (25th,75th)  | 11.1 (6-41.8) | 12.5 (5.7-14) |
| IL-10, mean (SD)          | 60.6 (47.3) | 16.8 (10.4) |
| Interventions, n (%)      | | |
| Glucocorticoids           | 46 (50) | 80 (39) |
| Anticoagulation therapy   | 33 (35.9) | 66 (32.2) |
| Mechanical ventilation    | 68 (74) | 104 (50.7) |
| Urinary catheterization   | 72 (78.3) | 83 (40.5) |
| Deep venous catheterization| 66 (71.7) | 74 (36.1) |
| Continuous renal replacement therapy | | |
| Blood transfusion          | 11 (12) | 19 (9.3) |
| LOS (days), median (25th,75th) | | |
| Hospital                  | 23.5 (12-34) | 22 (10-32.5) |
| ICU                       | 11 (7-17) | 11 (6-16.5) |
| Mortality, n (%)          | | |
| In-hospital   | 42 (45.7) | 52 (25.4) |
|--------------|-----------|-----------|
| 30-day       | 32 (34.8) | 48 (23.4) |
| 90-day       | 39 (42.4) | 52 (25.4) |

\(^a\) Other cardiovascular diseases included coronary heart disease, arrhythmia, myocardiosis and valvular heart disease.

\(^b\) Data of 89, 77 and 21 patients were available for HLA-DR expression at day 1, 3 and 7 respectively, in which 35, 34 and 33, 18 and 8 patients developed secondary infection. And data of 87, 38 and 18 patients were available for cytokines at day 1, 3 and 7 respectively, in which 33, 18 and 8 patients developed secondary infection.

\(^c\) In the group of secondary infection, it was referred to the interventions before the onset of secondary infection.

**Table 2. Characteristics of secondary infections**

| Site of infection \(^a\), n (%) |          |
|-------------------------------|----------|
| **Respiratory tract**         |          |
| PNU                           | 83 (55.3)|
| LUNG                          | 1 (0.7)  |
| **Urinary tract**             |          |
| SUTI                          | 41 (27.3)|
| OUTI                          | 1 (0.7)  |
| **Blood stream and disseminated infection** |          |
| LCBI                          | 12 (8)   |
| DI                            | 6 (4)    |
| **Abdomen**                   |          |
| IAB                           | 4 (2.7)  |
| GIT                           | 1 (0.7)  |
| **Skin and soft tissue**      |          |
| ST                            | 1 (0.7)  |
| **Time of onset of the first identified secondary infection** |          |
| Median (25th,75th)            | 8 (5.25,14)|
| Time range, n (%)             |          |
| day 3                         | 5 (5.4)  |
| > day 3, ≤day 7               | 36 (39)  |
| > day 7, ≤day 15              | 33 (35.9)|
| > day 15                      | 18 (19.6)|
| **Patients with multiple secondary infections, n (%)** | 26 (28.3) |
| **Secondary infection without identified pathogens, n (%)** | 23 (15.3) |

\(^a\) Diagnosis was according to CDC/NHSN criteria [25]. PNU Pneumonia, LUNG Other infections of the lower respiratory tract, SUTI Symptomatic urinary tract infection, OUTI Other infections of the urinary tract, DI Disseminated infection, GIT Gastrointestinal tract, IAB Intraabdominal infection, LCBI Laboratory-confirmed bloodstream infection, ST Soft tissue infection.
Table 3. Results of multivariate logistic regression test of the risk factors of secondary infection

| Variables                         | Partial regression coefficient | Standard error | Wald χ² |
|----------------------------------|-------------------------------|----------------|---------|
| Urinary catheterization          | 1.219                         | 0.325          | 14.109  |
| Deep venous catheterization      | 0.959                         | 0.309          | 9.601   |

*a Analysis was conducted by method Backward: Conditional. Variable blood transfusion was removed on step 2, mechanical ventilation on step 3, APACHE II score on step 5 and SOFA score on step 5.

Additional Files

Additional file 1: Figure S1. Illustration of multistate model to explore the expected length of stay. (JPEG)

Additional file 2: Table S1. Time of onset, pathogen and diagnostic criteria of secondary infection. (DOCX)

Additional file 3: Table S2. Results of the comparison of the change of HLA-DR expression and serum cytokines levels. (DOCX)

Additional file 4: Table S3. Characteristics of the septic patients classified according to the prognosis. (DOCX)

Additional file 5: Table S4. Results of multivariate logistic regression test of risk factors of the in-hospital death. (DOCX)

Additional file 6: Figure S2. Kaplan-Meier survival curves of septic patients after admission. (JPEG)

Additional file 7: Figure S3. Kaplan-Meier survival curves of septic patients after day 15. (JPEG)

Supplementary Figure Legends:

**Figure S1 Illustration of multistate model to explore the expected length of stay**

Patients without secondary infection would move from state 0 to state 2 or state 3.

Patients with secondary infection would move from state 0 to state 1, and then to state 2...
Figure S2 Kaplan-Meier survival curves of septic patients after admission

(A) Survival curves of overall septic patients before day 15; (B) Survival curves of septic patients without septic shock before day 15; (C) Survival curves of septic patients with septic shock before day 15; (D) Survival curves of overall septic patients before day 30; (E) Survival curves of septic patients without septic shock before day 30; (F) Survival curves of septic patients with septic shock before day 30; (G) Survival curves of septic patients without septic shock before day 90; (H) Survival curves of septic patients with septic shock before day 90.

Figure S3 Kaplan-Meier survival curves of septic patients after day 15

Cumulative survival rate was considered as 1 at day 15. (A) Survival curves of overall septic patients between day 15 and 30; (B) Survival curves of septic patients without septic shock between day 15 and 30; (C) Survival curves of septic patients with septic shock between day 15 and 30; (D) Survival curves of overall septic patients between day 15 and 90; (E) Survival curves of septic patients without septic shock between day 15 and 90; (F) Survival curves of septic patients with septic shock between day 15 and 90.

Figures
Figure 1

Study Flowchart.
Biomarkers of immune status in septic patients stratified according to developing secondary infection or not. Data of a part of patients were available for HLA-DR expression and cytokines. The exact numbers were shown in Table 1. Data were presented as median (shown as a triangle or circle) and 25- and 75- percentile error bars. Exceptions were mean and standard deviation error bars were used in HLA-DR expression at day 3 and IL-10 level at day 7. * P < 0.05, ** P < 0.01, *** P < 0.001. SI, secondary infection. NSI, non-secondary infection.
Figure 3

Representative plots of monocyte HLA-DR measurement by flow cytometry

Monocyte HLA-DR expression was measured by flow cytometry. The samples were collected at day 3 after admission. (A) The left dot-plot (SSC vs. FITC) delimited the monocytic region. The right dot-plot (APC vs. FITC) delimited the CD14+ HLA-DR+ monocyte (upper right region). The analysis was performed on a patient with immunosuppression as was reflected by the decreased proportion of CD14+ HLA-DR+ monocyte (18.5%). (B) The same strategy of analysis was used on a patient
without immunosuppression. FITC, fluorescein isothiocyanate. APC, allophtocyanin. SSC, side scatter.

Figure 4

Expected length of stay of septic patients with and without secondary infection

SI, secondary infection. NSI, non-secondary infection.
Kaplan-Meier survival curves of overall septic patients before day 90 SI, secondary infection. NSI, non-secondary infection.

Supplementary Files

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