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Using multistate model to estimate the impact of nosocomial infection on outcomes in burn patients in Southeast China

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Using multistate model to estimate the impact of nosocomial infection on outcomes in burn patients in Southeast China

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Abstract:

Objective: Due to the defects in skin barrier function and immune response, burn patients who survive the acute phase of a burn injury are at a high risk of nosocomial infection (NI). The aim of this study was to evaluate the impacts of NI on outcomes in burn patients using a multistate model. **Design and Setting:** A retrospective observational study was conducted in burn unit and intensive care unit (ICU) in the First Affiliated Hospital of Wenzhou Medical University, Wenzhou, China. **Participants:** Data were obtained from 1143 records of patients admitted with burn between 1 January 2013 to 31 December 2016. **Methods:** Risk factors for NIs were determined by binary logistic regression. The extended Cox model with time-varying covariates was used to determine the impact of NIs on mortality, and Cumulative incidence functions (CIF) were calculated. Multiple-linear regression analysis was applied to detect the variables associated with LOS. Using a multi-state model, the extra LOS due to NI were determined. **Results:** 15.8 percent of total burn patients were suffered from NIs and incidence density of NIs was 9.6 per 1000 patient-days. NIs significantly increased the rate of death (hazard ratio: 4.49, CI_{95} 2.309~8.722, P<0.001), and the cumulative probability of death for a patient with NI was greater that for a patient who remained free of NI after around day 7. The expected extra LOS due to NIs was 12.9 days (CI_{95} 6.8~19.0). **Conclusions:** Using appropriate statistical methods, the present study provided the further information about the impact of NIs on LOS and mortality in burn patients. Considering the consequences of NIs, surveillance and prevention are required to reduce the incidence of NIs, and then improve the outcomes of burns.

Key words: burn, nosocomial infection, length of stay, mortality, multi-state model

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Strengths and limitations of this study

1. Nosocomial infection was associated with the increased cumulative incidence of burn death.

2. The expected extra Length of stay due to nosocomial infections among burn patients was 14.6 days.

3. The present study was performed in a single center and the results need to be further confirmed by multiple center trials

4. Multi-state and competing risks analysis can be used to estimate the impact of nosocomial infections in burn patients.

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Introduction

Burn injury, as a common cause of morbidity and mortality, has been recognized as a global public health problem. According to the data from WHO, burns account for an estimated 300, 000 deaths each year [1]. Previous evidence illustrated that burn shock and inhalation injury were the major cause of early death among patients with burn injury [2,3]. Due to the advance in fluid resuscitation, surgical approach, organ function protection, antibiotic innovation and other adjunct strategies, the early mortality of burn patients decreased dramatically over the last 30 years [4,5]. On the other hand, because of the defects in skin barrier function and immune response, burn patients who survive the acute phase of a burn injury are at a high risk of acquiring nosocomial infection (NI) [6].

It has been reported that 30% to 80% of burn patients were suffered from NIs [7-9]. Nevertheless, the exact impact of NIs on the outcomes of burn patients remains elusive. A few studies reported the association between NIs and higher mortality after burn injury [7-9]. It should be noted that infection only can impact on LOS and outcomes after it has started [10,11]. So, appropriate statistical methods for estimating the risk of death and LOS due to NI among burn patients would be helpful in making medical decisions and developing policy. In the present study, using a multistate model, the impacts of NI on outcomes and LOS were determined among 1143 patients with burn.

Materials and methods

1. Patients

A retrospective study was conducted in burn unit and intensive care unit (ICU) in the First Affiliated Hospital of Wenzhou Medical University, Wenzhou, China. The

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burn unit has 72 beds and there are 50 beds in the ICU. After approval by the Institutional Review Board of the First Affiliated Hospital of Wenzhou Medical University, data of total 1143 patients admitted with burn were collected during January 2013 to December 2016. Exclusion criteria were as follows: (1) LOS≤ 48 hours; (2) admission to the hospital later than 3 days post burn. 157 burn patients were ineligible by exclusion criteria, and 986 patients were enrolled in the study. As the present study was an observational and retrospective study, informed consent was waived by the Medical Ethics Committee.

2. Management

Burn patients with acute respiratory failure or shock were admitted to the ICU, and they were transferred to the Burn Unit when the tracheal tube is removed or the hemodynamic situation of them becomes stable, according to the judgments of burn surgeon and ICU doctor. Patients without shock or acute respiratory failure were admitted to Burn Unit. Resuscitation were performed according to the modified Evans (Ruijin) formula as described by previous paper [12,13]. Dressings were changed every 1-3 days by doctors. Silver sulfadiazine were applied on deep partial-thickness and full-thickness burns. For full-thickness burns, early surgical excision of burn eschar and biological closure were performed when the patients' condition permits. Prophylactic antibiotic therapy was performed in patients with TBSA>30% or inhalation injury. In addition, patients with clinically suspected or confirmed infection were treated with antibiotics and adjusted according to the results of isolate's susceptibility.

3. Definitions and data collection

Patients with a history of smoke or fire exposure in a closed space or maxillofacial burn were suspected to have inhalation injury. The diagnosis was confirmed by physical findings including changes in voice and carbonaceous sputum production or by fiberoptic bronchoscopy [14].

NI in burn patients was defined as infection occurring 48 hours after hospital admission. There were four main types of NIs (burn wound infection, bloodstream

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infection, pneumonia, urinary tract infection) according to the criteria of the Centers for Disease Control and Prevention (CDC, 2008) [15].

The characteristics of NI including time, site and pathogen were recorded. For patients with NI at the same site, only the first episode of it was analyzed. Additionally, the following data were collected: gender, age, history of diabetes, date of admission, burn types (flame, scalding, electric and others), burn size and depth, inhalation injury as well as dates of discharge and death.

4. Statistical Analysis

Data are presented as a percentage of a percentage of total or interquartile ranges ($25t^{h}$ and 75^{th} percentiles), as appropriate. Mann-Whitney U test is used to analysis continuous variables while categorical variables were analyzed by the Chi-square test. Univariate analysis was performed to assess the potential variables associated with NI and mortality. The variables with p-value less than 0.05 were used for further analysis. The strength of a link between two variables was determined by Spearman's Rank Correlation Coefficient. If the high correlations (>0.4) between two variables was observed, one of them would be removed. Multivariate binary logistic regression analyses and Cox model were used to determine the risk factors for NI and death, respectively. In Cox model, NI was modeled as a time-varying covariate by the 'survival' package in R. Cumulative incidence functions were calculated by the "cmprsk" package. Additionally, linear regression analysis was applied to detect the variables associated with hospital LOS. The 'etm' package in R was performed to calculate the difference in length of stay between patients with and without NI. There are four states in our multistate model: admission, NI, discharge alive and death. The detail information about this multistate model were shown in Figure 1.

R 3.4.1 software and SPSS 18.0 were used to prepare and analysis the data. Statistical significance was expressed as both p values and 95% confidence intervals (CI_{95}). A two-sided p-value <0.05 was considered statistically significant.

Results:

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1. Patient characteristics

During the study period, a total of 1143 burn patients were admitted to the hospital, and 986 patients who met the criteria were included in the final analysis. Demographic and burn-related characteristics are shown in Table 1. 65.1 percent of the patients were men and 34.9% were women. The median age was 37 (interquartile range [IQR], 18-49) years and 7.1% were elderly patients (65 years and older). 47.6% of the patients had < 10% TBSA burn, 30.8% had 10-29% TBSA burn, and 21.6% of burn patients with TBSA more than 30%. The main burn type is flame (78.2%), followed by scalding (9.7%), electric (7.4%) and other types (4.7%). There were 46 (4.7%) patients had inhalation injury. The hospital morality was 5.5% (54/986) and the median length of hospital stay was 14 (IQR 8-28).

2. Characteristics of NIs

156 burn patients had 209 NIs, and the median time from admission to the NI was 7 days (IQR 5-10). Over all NI rate was 9.6 per 1000 patient-days. Among all NIs, burn wound infection (BWI) was the most frequent infection (45.9%), followed by blood stream infection (BSI) (24.8%), pneumonia (23.4%) and urinary tract infection (UTI) (5.7%) (Fig.2A). As shown in Fig.2B, a total 237 microorganisms were isolated. The most common pathogens was *Acinetobacter baumannii* (30.8%), followed by *Pseudomonas aeruginosa* (21.5%), *klebsiella pneumoniae* (16.9%) and *Staphylococcus spp* (11%) (Fig.2B).

Univariate analysis indicated that there were significant differences in age, the percentage of patients more than 65 years old, diabetes, TBSA<10%, TBSA>50%, full thickness burn, inhalation injury and LOS between patients with and without Nis (Table 1). In multiple logistic regression, there was a statistically significant increased odds ratio for NI in patients with diabetes (odds ratio [OR] 1.745; 95% confidence interval [CI_{95}] 1.476~1.745, p<0.001) and a longer LOS in the hospital (OR 1.023; CI_{95} 1.022~1.024, p<0.001), burn injuries more than 50% TBSA (OR 2.569; CI_{95} 2.312~2.856, p<0.001), full thickness burn (OR 2.124; CI_{95} 2.312~2.856, p<0.001),

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and inhalation injury (OR 8.051; CI_{95} 6.993~9.268, p<0.001) (Table 2).

3. Impact of NIs on hospital death of burn patients

As shown in Table 1, the hospital mortality of patients with and without NI were 16.0% and 3.5%, respectively. Univariate analysis indicated that the mortality of patients with NI, TBSA more than 30%, full thickness burn, and inhalation injury were higher than patients without (Table S1). Additionally, the mortality of patients with TBSA less than 10% was lower than patients with burn injuries more than 10%. Using a Cox regression model with NI modeled as a time-varying covariate, we found the risk of hospital death for patients with NI was 4.49 times higher than that for patients without it (hazard ratio:4.49, CI_{95} 2.309~8.722, P<0.001) (Table 2). Death was rarer in burn patients with TBSA<10%, while patients with inhalation injury were more likely to die. Cumulative incidence functions for death were shown in Fig 3A. The cumulative probability of discharge was consistently lesser for an infected patient (left panel). As shown in Fig 3B, the cumulative probability of death for a patient with NI was greater that for a patient who remained free of NI after around day 7 (right panel).

4. Extra length of stay

As shown in Fig.1, the median LOS for patients without NI was 13 days (IQR 7-24). For patients with NI, the median LOS was 27 days (IQR 13.25-57.75). Because the LOS distribution is positively skewed, the logarithm (base 10) of LOS was used as the response variable in multiple linear regressions. Based on the results of multiple linear regressions, variables associated with LOS were inhalation injury, TBSA30-50%, TBSA>50%, electric burn, flame burn, full thickness burn as well as NI (Table 3). Using a multi-state model, the expected extra length of stay due to NI was 12.9 days [CI_{95} 6.8~19.0, standard error (SE): 3.11, P<0.001] (Fig. 4).

Discussion:

Burn patients, especially those with severe burns, are at high risk for local and systemic infections. Although infection control program has been performed in most

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burn centers and hospitals, the incidence of NI remain high until now. Alp E et al. reported 11 percent of total burn patients were suffered from NI and incidence density was 14.7 per 1000 patient days [10]. In patients who have > 20% TBSA burn and need for surgical intervention, the incidence of NI was 70% [16]. In the present study, incidence density of NI was 9.6 per 1000 patient days which was less than the rate reported by Alp E et al. and Jeschke MG et al. BWIs was the most common infections in our burn center. *A. baumannii* and *P. aeruginosa* accounted for about 50% of total isolates, and *A. baumannii* was the predominant pathogen. Previously study illustrated that *A. baumannii* was the most common Gram-negative pathogen isolated from burn patients [9]. According to the data published by Alp E et al [10], 57% of isolates from burns was *A. baumannii* in 2009. As *A. baumannii* is increasingly implicated as a main cause of NI, more strict measures have been taken to reduce the incidence of *A. baumannii* infection, especially in Asian countries [17].

Many factors are associated with the incidence of NI in burns, including burn injury induced immunosuppression [6, 8-10]. Clinical and experimental evidence illustrated that severe systemic inflammation after burn injuries can result in a compensatory down-regulation of immune responses, which is characterized by decreased number of T helper lymphocytes, increased suppressive activity of $Foxp3^{+}T$ cells as well as elevated levels of anti-inflammatory cytokines[18-20]. The immune response declines in efficiency with age, and increased susceptibility to infection was also observed by previous studies [21,22]. The present study showed that burn patients older than 65 years were more susceptible to NI. Similar to the previous reports, TBSA, Full thickness burn, inhalation injury and LOS were observed to be associated with NI [8-10].

Burn size was a main negative risk factor for death [16, 23-24]. The lower death rate of patients with TBSA<10% was observed in the present study which was consist with the results of other studies [23-24]. Inhalation injury usually cause pulmonary and systemic complications which greatly increases the risk of death after burn and the results of our study confirmed this [23-24]. It has been reported that, in patients

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with more than 40% TBSA, over 70% of all deaths were related to sepsis resulting from BWIs and other infection complications [6,8-10,25]. In a study of 11,793 burn patients, the mortality in the BSI group was higher than the control group (21.9% *vs* 4.2%, OR=6.041) [26]. In the present study, NI was modeled as a time-varying factor in a competing risk model, we found the risk of hospital death for burns with NI was 4.49 times higher than that for patients without it, and the cumulative probability of discharge was consistently lesser for an infected patient. So, effective NI prevention and control measures may help to improve survival in burn patients.

The association between NI and LOS has been illustrated by many studies. The median LOS was about 2-fold higher in trauma patients with NI compared with patients without infection [27]. Among patients with critical illness, NI increased the LOS by approximately 18 days per patients [28]. NI after burn has been considered as a risk factor for prolonged LOS, although clinical data are still lacking. Shupp JW et al. reported that BSI was associated with longer hospital LOS in burn patients [26]. Nevertheless, NI can only impact LOS after it has started and the duration of hospitalization prior to the infection should be controlled. So, a multistate model was used in the present study to estimation of extra LOS caused by NI. We found that the expected extra length of stay due to NI in burn patients was 12.9 days.

There are some limitations in the present study. First, efforts used to prevent NI, such as antibiotic treatment and surgery, may have been started before the diagnosis was made. So, our assessment of the impact of NI on LOS and mortality should be regarded as a lower estimate. Second, as an observational and retrospective study, some potential factors, such as nursing protocols and the use of anti-peptic ulcer or immunosuppression drugs, that may be associated with NI and outcomes were not available. These factors need to be taken into consideration in the prospective studies. Additionally, the present study was performed in a single center and the results need to be further confirmed by multiple center trials. Nevertheless, the present study provided additional information about the impact of NI on LOS and mortality in burn patients. The model used in the present study may help to improve the accuracy of

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estimates of outcomes due to NIs in burns.

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Conflict of interest:

The authors declare that they have no completing interests

Authors' contributions

HLG and ZJL wrote the protocol, participated in the data analysis, and contributed to writing this manuscript. GJZ, XWL, JJX and CJL collected the data. All authors have read and approved the final manuscript.

Ethics approval: Institutional Review Board of the First Affiliated Hospital of Wenzhou Medical University.

Provenance and peer review: Not commissioned; externally peer reviewed.

Data sharing statement: No additional data are available.

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Figure legends

Figure.1 Multistate model. Our model including four states: admission, nosocomial infection, discharge alive and death. After admission, patients may be infected or not, then they may be discharge alive or die.

Figure.2 Characteristics of nosocomial infections. BWI: Burn wound infection; BSI: blood stream infection; PI: pulmonary infection; UTI: urinary tract infection.

Figure.3 Cumulative incidence functions for discharge (A) and death (B) in burn patients. read lines: nosocomial infection; black lines: no nosocomial infection.

Figure 4. Expected extra length of stay in patients without (red line) and with (black line) infection. LOS: length of stay. NI: nosocomial infection

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Table 1. Demographics and clinical characteristic of burn patients with and without nosocomial infection.

Variables	Total n=986	NI n=156	No-NI n=830	*P* value
Male, n(%)	642 (65.1%)	105 (64.7%)	537 (67.3%)	0.530
Age (years), median (25th, 75th)	37 (18,49)	37 (17,49)	37 (24,37)	0.470
≥65 years, n(%)	70 (7.1%)	20 (12.8%)	50 (6.0%)	**0.002**
Diabetes, n (%)	38 (3.9%)	11 (7.1%)	27 (3.3%)	**0.024**
TBSA, n (%)				
<10%	469 (47.6%)	55 (35.3%)	414 (49.9%)	**0.031**
10-29%	304 (30.8%)	38 (24.4%)	266 (32.0%)	0.056
30-50%	143 (14.5%)	23 (14.7%)	120 (14.5%)	0.926
>50%	70 (7.1%)	40 (25.6%)	30 (3.6%)	**<0.001**
Full thickness burn, n (%)	221 (22.4%)	60 (38.5%)	161 (19.4%)	**<0.001**
Inhalation injury, n (%)	46 (4.7%)	38 (24.3%)	8 (1.0%)	**<0.001**
Burn type, n (%)				
flame	771 (78.2%)	118 (75.6%)	653 (84.7%)	0.4
scalding	96 (9.7%)	11 (7.1%)	85 (10.2%)	0.218
electric	73 (7.4%)	15 (9.6%)	58 (7.0%)	0.25
others	46 (4.7%)	12 (7.7%)	34 (4.1%)	0.051

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Length of hospital stay				
median (25th, 75th)	14 (8, 28)	27 (13.25, 57.75)	13 (7, 24)	**<0.001**
In-hospital mortality, n (%)	54 (5.5%)	25 16.0	29 3.5	**<0.001**

NI: nosocomial infection; TBSA: total body surface area

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Table.2 Results of the Cox-proportional hazard analysis of nosocomial infection and hospital death

Outcomes	Variables	HR	CI_{95}	P value
NI	Age>65 years	1.906	1.185~3.067	0.008
	TBSA>50%	1.812	1.112~2.953	0.017
	Full thickness burn	1.897	1.355~2.657	<0.001
	Inhalation injury	3.094	2.004~4.777	<0.001
	Length of stay	1.006	1.001~1.010	0.019
Death	Nosocomial infection	4.488	2.209~8.722	<0.001
	TBSA<10%	0.191	0.070~0.519	0.001
	Inhalation injury	3.332	1.683~6.985	<0.001

NI: nosocomial infection; TBSA: total body surface area; HR: hazard ratio; CI_{95}: 95% confidence interval

Table.3 Results of multiple linear regressions analysis of length of stay (days)

Variables	B	CI_{95}	P value
TBSA 30-50%	0.153	0.091~0.215	<0.001
TBSA >50%	0.259	0.170~0.347	<0.001
Full thickness burn	0.109	0.057~0.161	<0.001
Electric burn	0.204	0.107~0.302	<0.001
Flame burn	0.092	0.031~0.153	<0.003
Nosocomial infection	0.218	0.155~0.280	<0.001

TBSA: total body surface area; CI_{95}: 95% confidence interval

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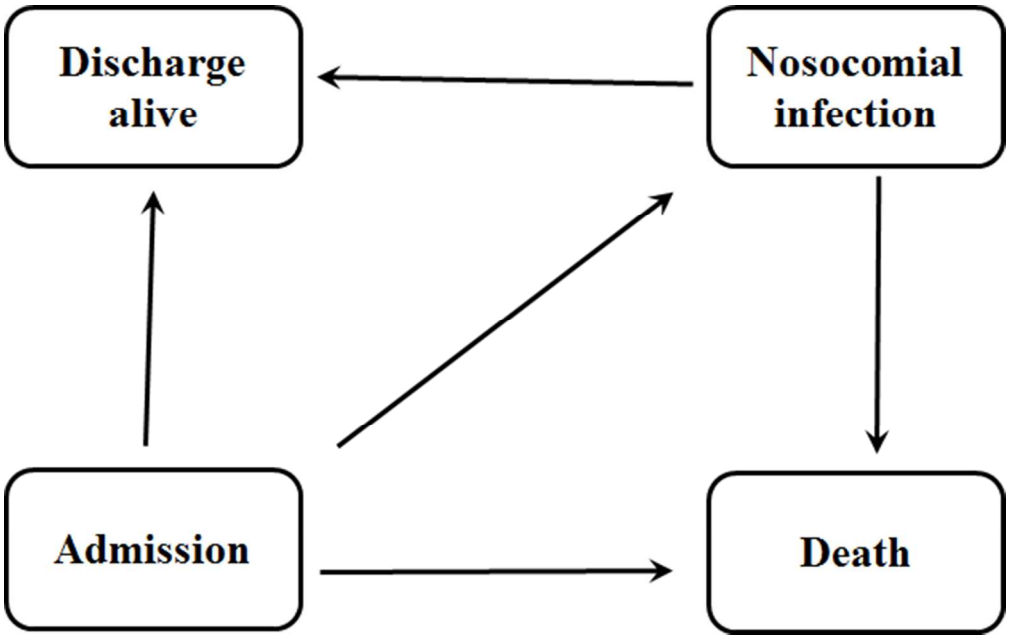

Figure.1 Multistate model.

220x138mm (300 x 300 DPI)

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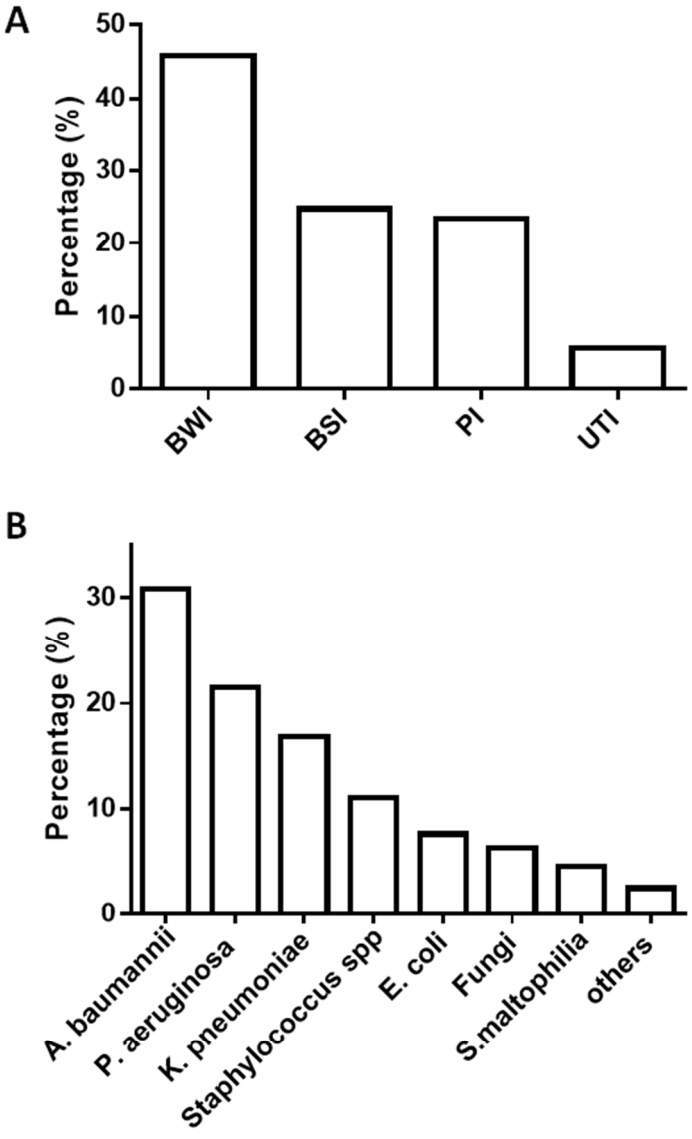

Figure.2 Characteristics of nosocomial infections.

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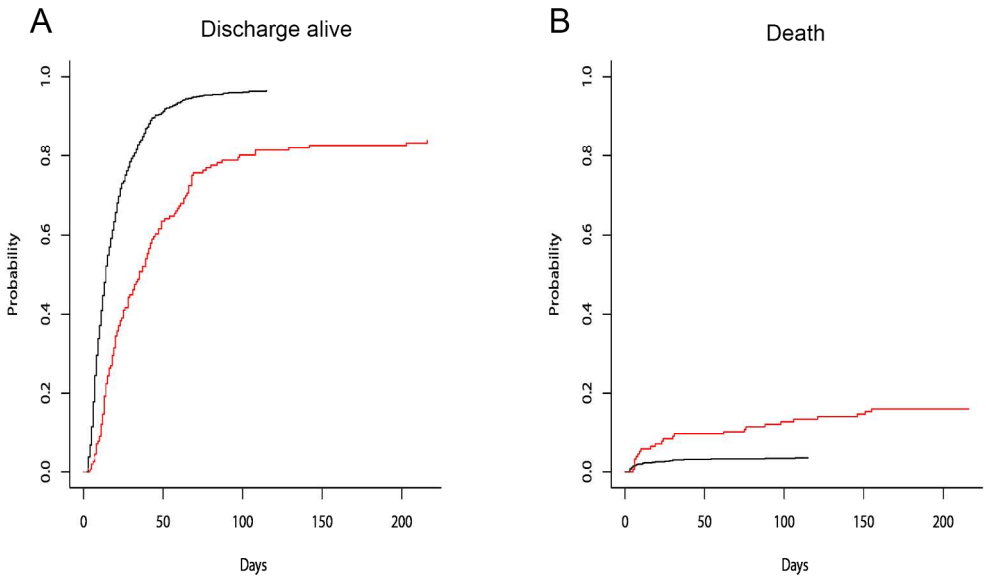

Figure.3 Cumulative incidence functions for discharge (A) and death (B) in burn patients.

190x127mm (300 x 300 DPI)

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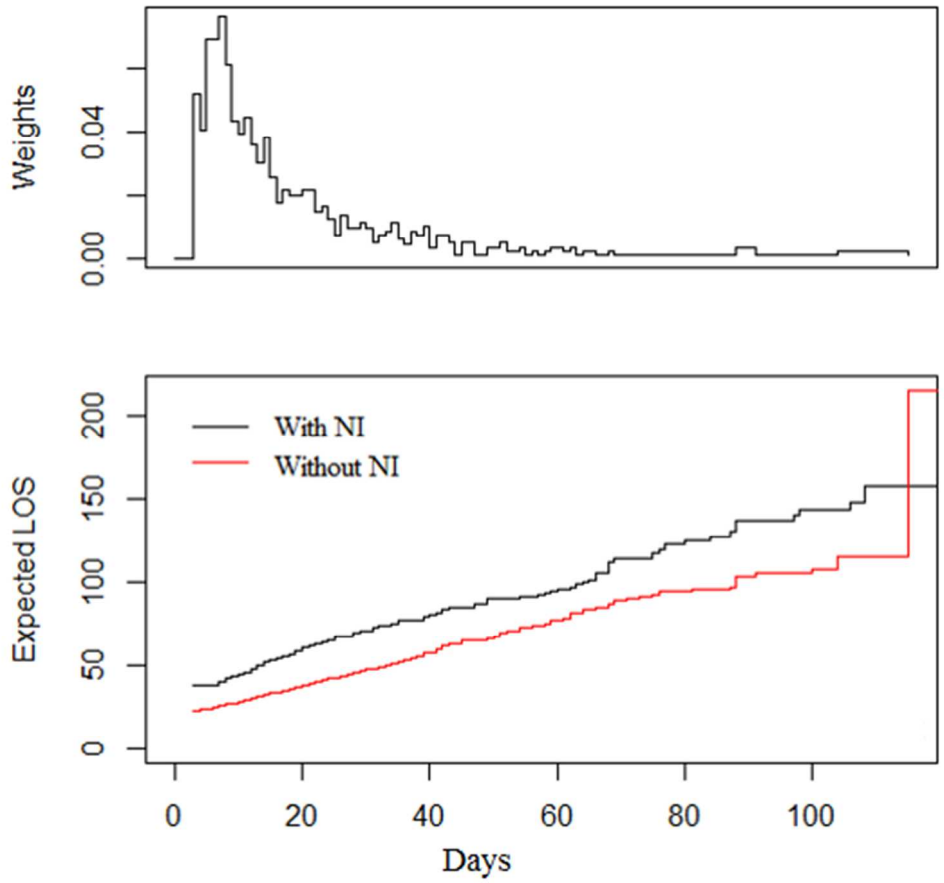

Figure 4. Expected extra length of stay in patients without (red line) and with (black line) infection.

275x275mm (300 x 300 DPI)

The STROBE-Vet statement checklist.

	Item	STROBE-Vet recommendation	Page #
Title and Abstract	1	(a) Indicate that the study was an observational study and, if applicable, use a common study design term	2
		(b) Indicate why the study was conducted, the design, the results, the limitations, and the relevance of the findings	2-3
Background / rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	(a) State specific objectives, including any primary or secondary prespecified hypotheses or their absence	4
		(b) Ensure that the level of organization[a] is clear for each objective and hypothesis	4
Study design	4	Present key elements of study design early in the paper	4-5
Setting	5	(a) Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4-5
		(b) If applicable, include information at each level of organization	
Participants [b]	6	(a) Describe the eligibility criteria for the owners/managers and for the animals, at each relevant level of organization	5
		(b) Describe the sources and methods of selection for the owners/managers and for the animals, at each relevant level of organization	5
		(c) Describe the method of follow-up	
		(d) For matched studies, describe matching criteria and the number of matched individuals per subject (e.g., number of controls per case)	
Variables	7	(a) Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. If applicable, give diagnostic criteria	5-6
		(b) Describe the level of organization at which each variable was measured	5-6
		(c) For hypothesis-driven studies, the putative causal-structure among variables should be described (a diagram is strongly encouraged)	

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Data sources / measurement	8*	(a) For each variable of interest, give sources of data and details of methods of assessment (measurement). If applicable, describe comparability of assessment methods among groups and over time	6
		(b) If a questionnaire was used to collect data, describe its development, validation, and administration	
		(c) Describe whether or not individuals involved in data collection were blinded, when applicable	
		(d) Describe any efforts to assess the accuracy of the data (including methods used for "data cleaning" in primary research, or methods used for validating secondary data)	6
Bias	9	Describe any efforts to address potential sources of bias due to confounding, selection, or information bias	
Study size	10	(a) Describe how the study size was arrived at for each relevant level of organization	6
		(b) Describe how non-independence of measurements was incorporated into sample-size considerations, if applicable	
		(c) If a formal sample-size calculation was used, describe the parameters, assumptions, and methods that were used, including a justification for the effect size selected	
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why	6
Statistical methods	12	(a) Describe all statistical methods for each objective, at a level of detail sufficient for a knowledgeable reader to replicate the methods. Include a description of the approaches to variable selection, control of confounding, and methods used to control for non-independence of observations	6
		(b) Describe the rationale for examining subgroups and interactions and the methods used	
		(c) Explain how missing data were addressed	
		(d) If applicable, describe the analytical approach to loss to follow-up, matching, complex sampling, and multiplicity of analyses	
		(e) Describe any methods used to assess the robustness of the analyses (e.g., sensitivity analyses or quantitative bias assessment)	
Participants	13*	(a) Report the numbers of owners/managers and animals at each stage of study and at each relevant level of organization - e.g., numbers eligible, included in the study, completing follow-up, and analyzed	6-7

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		(b) Give reasons for non-participation at each stage and at each relevant level of organization	
		(c) Consider use of a flow diagram and/or a diagram of the organizational structure	
Descriptive data on exposures and potential confounders	14*	(a) Give characteristics of study participants (e.g., demographic, clinical, social) and information on exposures and potential confounders by group and level of organization, if applicable	7
		(b) Indicate number of participants with missing data for each variable of interest and at all relevant levels of organization	
		(c) Summarize follow-up time (e.g., average and total amount), if appropriate to the study design	
Outcome data	15*	(a) Report outcomes as appropriate for the study design and summarize at all relevant levels of organization	7
		(b) For proportions and rates, report the numerator and denominator	
		(c) For continuous outcomes, report the number of observations and a measure of variability	
Main results	16	(a) Give unadjusted estimates and, if applicable, adjusted estimates and their precision (e.g., 95% confidence interval). Make clear which confounders and interactions were adjusted. Report all relevant parameters that were part of the model	7-8
		(b) Report category boundaries when continuous variables were categorized	
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done,-such as sensitivity/robustness analysis and analysis of subgroups	
Key results	18	Summarize key results with reference to study objectives	3,10
Strengths and Limitations	19	Discuss strengths and limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	3,10
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	10
Generalizability	21	Discuss the generalizability (external validity) of the study results	8-10
~~Funding~~ Transparency	22	(a) Funding- Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based (b) Conflicts of interest-Describe any conflicts of interest, or lack thereof, for each author	11

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		(c) Describe the authors' roles- Provision of an authors' declaration of transparency is recommended (d) Ethical approval- Include information on ethical approval for use of animal and human subjects (e) Quality standards-Describe any quality standards used in the conduct of the research	

[a] Level of organization recognizes that observational studies in veterinary research often deal with repeated measures (within an animal or herd) or animals that are maintained in groups (such as pens and herds); thus, the observations are not statistically independent. This non-independence has profound implications for the design, analysis, and results of these studies.
[b] The word "participant" is used in the STROBE statement. However, for the veterinary version, it is understood that "participant" should be addressed for both the animal owner/manager and for the animals themselves.
*Give such information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

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Using competing risk and multistate model to estimate the impact of nosocomial infection on length of stay and mortality in burn patients in Southeast China

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Using competing risk and multistate model to estimate the impact of nosocomial infection on length of stay and mortality in burn patients in Southeast China

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Abstract:

Objective: Due to the defects in skin barrier function and immune response, burn patients who survive the acute phase of a burn injury are at a high risk of nosocomial infection (NI). The aim of this study was to evaluate the impacts of NI on length of stay (LOS) and hospital mortality in burn patients using a multistate model. **Design and Setting:** A retrospective observational study was conducted in burn unit and intensive care unit (ICU) in the First Affiliated Hospital of Wenzhou Medical University, Wenzhou, China. **Participants:** Data were obtained from 1143 records of patients admitted with burn between 1 January 2013 to 31 December 2016. **Methods:** Risk factors for NIs were determined by binary logistic regression. The extended Cox model with time-varying covariates was used to determine the impact of NIs on hospital mortality, and Cumulative incidence functions (CIF) were calculated. Multiple-linear regression analysis was applied to detect the variables associated with LOS. Using a multi-state model, the extra LOS due to NI were determined. **Results:** 15.8 percent of total burn patients were suffered from NIs and incidence density of NIs was 9.6 per 1000 patient-days. NIs significantly increased the rate of death (hazard ratio: 4.30, CI_{95} 2.229~8.298, P<0.001). The cumulative probability of death for patients with NI was greater that for those without NI. The extra LOS due to NIs was 17.68 days (CI_{95} 11.31~24.05). **Conclusions:** Using appropriate statistical methods, the present study further illustrated that NIs was associated with the increased cumulative incidence of burn death and increased LOS in burn patients.

Key words: burn, nosocomial infection, length of stay, mortality, multi-state model

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Strengths and limitations of this study

1. Using Multi-state and competing risks analysis, the present study assessed the impact of nosocomial infections on hospital mortality and length of stay in burn patients.

2. Some potential factors, such as nursing protocols and the history of immunosuppression drugs, that may be associated with NI, length of stay (LOS) and mortality were not recorded.

3. This study was performed in a single center and the results need to be further confirmed by multiple center trials.

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Introduction

Burn injury, as a common cause of morbidity and mortality, has been recognized as a global public health problem. According to the data from World Health Organization (WHO), burns account for an estimated 300, 000 deaths each year [1]. Previous evidence illustrated that burn shock and inhalation injury were the major cause of early death among patients with burn injury [2,3]. Due to the advance in fluid resuscitation, surgical approach, organ function protection, antibiotic innovation and other adjunct strategies, the early mortality of burn patients decreased dramatically over the last 30 years [4,5]. On the other hand, because of the defects in skin barrier function and immune response, burn patients who survive the acute phase of a burn injury are at a high risk of acquiring nosocomial infection (NI) [6].

It has been reported that about 30-80% of burn patients suffered from NIs [7-9]. Nevertheless, the exact impact of NIs on the LOS and mortality of burn patients remains elusive. Williams *et al* [10] investigated the predominant causes of death in burned pediatric patients. They found that infection is the leading cause of death after burn injury. A recent study reported an incidence density of 14.7 infections/1000 patient days in burn patients and NIs was not the most significant risk factors for mortality [11]. It should be noted that NI is a time-varying factor, and it can develop at any time after admission. NIs can impact on length of stay (LOS) and mortality only after they have started [12,13]. So, appropriate statistical methods for estimating the risk of death and LOS due to NI among burn patients would be helpful in making medical decisions and developing policy. The aim of this study was to determine the impacts of NI on length of stay (LOS) and hospital mortality in burn patients using a multistate model.

Materials and methods

1. Patients

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A retrospective study was conducted in burn unit and intensive care unit (ICU) in the First Affiliated Hospital of Wenzhou Medical University, Wenzhou, China. The burn unit has 72 beds and there are 50 beds in the ICU. After approval by the Institutional Review Board of the First Affiliated Hospital of Wenzhou Medical University, data of total 1143 patients admitted with burn were collected during January 2013 to December 2016. Inclusion criteria: (1) age of 0-99 years; (2) admission to hospital no later than 3 days post-burn; (3) LOS>48 hours. As the present study was an observational and retrospective study, informed consent was waived by the Medical Ethics Committee.

2. Definitions and data collection

NI in burn patients was defined as infection occurring 48 hours after hospital admission. There were four main types of NIs (burn wound infection, bloodstream infection, pneumonia, urinary tract infection) according to the criteria of the Centers for Disease Control and Prevention (CDC) [14]. A case of burn wound infection was defined as a patient has change in burn wound appearance or character or patient with a burn has at least two of the following without other recognized cause: fever [Temperature (T) >38°C], hypothermia (T<36°C), hypotension, oliguria (<20 cm^2/hr), hyperglycemia at previously tolerated levels of dietary carbohydrate, or mental confusion, in which histologic examination of burn biopsy shows invasion of organisms into adjacent viable tissue. Bloodstream infection patient had at least one of the following clinical signs or symptoms with no other recognized cause: Fever (T>38°C), hypotension (systolic pressure ≤90 mm Hg), or oliguria (<20 cm^3/h); blood culture not done or no organisms or antigen detected in blood; and no apparent infection at another site and physician instituted treatment for sepsis. Patients had rales or dullness to percussion on physical examination of the chest or a chest radiographic examination that showed new or progressive infiltrate or consolidation, cavitation, or pleural effusion and new onset of purulent sputum or change in character of sputum were diagnosed with pneumonia. Finally, urinary tract infection patient with the following signs or symptoms with no other recognizable cause: fever

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(T>38°C), urgency, frequency, dysuria, or suprapubic tenderness; and at least one of the following: 1) positive dipstick for leukocyte esterase and/or nitrate; 2) physician diagnosis of urinary tract infection.

Patients with a history of smoke or fire exposure in a closed space or maxillofacial burn were suspected to have inhalation injury. The diagnosis of inhalation injury was made if the suspected patients had physical findings including changes in voice and carbonaceous sputum production, or had bronchoscopic evidence [15].

The characteristics of NI including time, site and pathogen were recorded. For patients with NI at the same site, only the first episode of it was analyzed. the potential factors which are associated with NIs, LOS and mortality were collected, including gender, age, history of diabetes, date of admission, burn types (flame, scalding, electric and others), burn size and depth and inhalation injury [7-9]. Additionally, the dates of discharge and death.

3. Management

Resuscitation were performed according to the modified Evans (Ruijin) formula as described by previous paper [16,17]. Dressings were changed every 1-3 days by doctors. Silver sulfadiazine were applied on deep partial-thickness and full-thickness burns. For full-thickness burns, early surgical excision of burn eschar and biological closure were performed when the patients' condition permits. Prophylactic antibiotic therapy was performed in patients who needing surgical intervention (perioperative period of debridement or auto skin grafting) or requiring mechanical ventilation. The strategy of Prophylactic antibiotic therapy was mainly based on the advice of doctors from the department of microbiology and infectious diseases and the previous antibiotic susceptibility pattern of the center. Additionally, patients met CDC criteria or with identified pathogens were treated with antibiotics and adjusted according to the results of isolate's susceptibility.

4. Statistical Analysis

Data are presented as a percentage of a percentage of total or interquartile ranges

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(25t^{h} and 75th percentiles), as appropriate. Mann-Whitney U test is used to analysis continuous variables while categorical variables were analyzed by the Chi-square test. Univariate analysis was performed to assess the potential variables associated with NI and hospital mortality. Variables included in the univariate analysis were age, sex, diabetes, burn types (flame, scalding, electric and others), TBSA (<10%, 10-29%, ≥30%), full thickness of burn, and inhalation injury. The variables with p-value less than 0.05 were used for further analysis. Cox model were used to determine the risk factors for NI and death. In Cox model, NI was modeled as a time-varying covariate by the 'survival' package in R. Cumulative incidence functions were calculated by the "cmprsk" package. Additionally, linear regression analysis was applied to detect the variables associated with hospital LOS. The 'etm' package in R was performed to calculate the difference in length of stay between patients with and without NI. There are four states in our multistate model: admission, NI, discharge alive and death. The detail information about this multistate model were shown in Figure 1.

R 3.4.1 software and SPSS 18.0 were used to prepare and analysis the data. Statistical significance was expressed as both p values and 95% confidence intervals (CI_{95}). A two-sided p-value <0.05 was considered statistically significant.

Results:

1. Patient characteristics

During the study period, a total of 1143 burn patients were admitted to the hospital. 157 burn patients were ineligible by exclusion criteria, and 986 patients were included in the final analysis. Demographic and burn-related characteristics are shown in Table 1. 65.1 percent of the patients were men and 34.9% were women. The median age was 37 (interquartile range [IQR], 18-49) years and 7.1% were elderly patients (65 years and older). 47.6% of the patients had < 10% TBSA burn, 30.8% had 10-29% TBSA burn, and 21.6% of burn patients with TBSA more than 30%. The main burn type is flame (78.2%), followed by scalding (9.7%), electric (7.4%) and other types (4.7%). There were 46 (4.7%) patients had inhalation injury. The hospital

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morality was 5.5% (54/986) and the median length of hospital stay was 14 (IQR 8-28).

2. Characteristics of NIs

156 burn patients had 209 NIs, and the median time from admission to the NI was 7 days (IQR 5-10). Over all NI rate was 9.6 per 1000 patient-days. Among all NIs, burn wound infection (BWI) was the most frequent infection (45.9%), followed by blood stream infection (BSI) (24.8%), pneumonia (23.4%) and urinary tract infection (UTI) (5.7%) (Fig.2A). As shown in Fig.2B, a total 237 microorganisms were isolated. The most common pathogens was *Acinetobacter baumannii* (30.8%), followed by *Pseudomonas aeruginosa* (21.5%), *klebsiella pneumoniae* (16.9%) and *Staphylococcus spp* (11%) (Fig.2B).

Univariate analysis indicated that there were significant differences in age, the percentage of patients more than 65 years old, diabetes, TBSA<10%, TBSA≥30%, full thickness burn, inhalation injury and LOS between burn patients with and without NIs (Table 1). Using a Cox regression model, there was a statistically significant increased odds ratio for NI in patients with age>65 years (HR 1.863; CI_{95} 1.135~3.060, p=0.014), full thickness burn (HR 1.721; CI_{95} 1.235~2.398, p<0.001), and inhalation injury (OR 3.618; CI_{95} 2.378~5.505, p<0.001) (Table 2).

3. Impact of NIs on hospital death of burn patients

As shown in Table 1, the hospital mortality of patients with and without NI were 16.0% and 3.5%, respectively. Univariate analysis indicated that the hospital mortality of patients with NIs were higher than those without NIs (Table S1). Using a Cox regression model with NI modeled as a time-varying covariate, we found the risk of hospital death for patients with NI was 5.92 times higher than that for patients without it (CI_{95} 3.098~11.31, P<0.001). After adjusting for TBSA and inhalation injury, the risk of hospital death for patients with NI was 4.30 times higher than for patients without NI (CI_{95} 2.229~8.298, P<0.001) (Table 2, Table S1). Cumulative incidence functions for death were shown in Fig 3A. The cumulative probability of discharge was consistently lesser for an infected patient (left panel). As shown in Fig 3B, the

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cumulative probability of death for a patient with NI was greater that for a patient without NI (right panel).

4. Extra length of stay

As shown in Fig.1, the median LOS for patients without NI was 13 days (IQR 7-24). For patients with NI, the median LOS was 27 days (IQR 13.25-57.75). Because the LOS distribution is positively skewed, the logarithm (base 10) of LOS was used as the response variable in multiple linear regressions. Based on the results of multiple linear regressions, NI was associated with LOS in burn patients. Other variables associated with LOS were TBSA, electric burn, flame burn, full thickness (Table 3). Using a multi-state model, the extra length of stay due to NI was 17.68 days [CI_{95} 11.31~24.05, standard error (SE): 3.25, P<0.001] (Fig. 4).

Discussion:

Burn patients are at high risk for local and systemic infections. Although infection control program has been performed in most burn centers and hospitals, the incidence of NI remain high until now. Alp E et al. reported 11 percent of total burn patients were suffered from NI and incidence density was 14.7 per 1000 patient days [11]. In patients who have > 20% TBSA burn and need for surgical intervention, the incidence of NI was 70% [18]. In the present study, incidence density of NI was 9.6 per 1000 patient days which was less than the rate reported by Alp E et al. and Jeschke MG et al. BWIs was the most common infections in our burn center. *A. baumannii* and *P. aeruginosa* accounted for about 50% of total isolates, and *A. baumannii* was the predominant pathogen. Previously study illustrated that *A. baumannii* was the most common Gram-negative pathogen isolated from burn patients [9]. According to the data published by Alp E et al [11], 57% of isolates from burns was *A. baumannii* in 2009. As *A. baumannii* is increasingly implicated as a main cause of NI, more strict measures have been taken to reduce the incidence of *A. baumannii* infection, especially in Asian countries [19].

Many factors contribute to the incidence of NI in burns, including burn injury

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induced immunosuppression [6-9]. Clinical and experimental evidence illustrated that severe systemic inflammation after burn injuries can result in a compensatory down-regulation of immune responses, which is characterized by decreased number of T helper lymphocytes, increased suppressive activity of $Foxp3^{+}$T cells as well as elevated levels of anti-inflammatory cytokines[20-22]. The immune response declines in efficiency with age, and increased susceptibility to infection was also observed by previous studies [12,23]. The present study showed that burn patients older than 65 years were more susceptible to NI. Similar to the previous reports, full thickness burn and inhalation injury were observed to be associated with NI [8,9,11].

The most notable finding in this study was the association between NIs and hospital mortality in burn patients. It has been reported that, in patients with more than 40% TBSA, over 70% of all deaths were related to sepsis resulting from BWIs and other infection complications [6,8,9,11,24]. Nevertheless, some evidence illustrated that NIs were not the main factor of death in overall burn patients. The different severity of burn injury and statistical method may contribute to the different results. In the present study, overall burn patients during the study period were enrolled, and NI was modeled as a time-varying factor in a competing risk model. The results illustrated that the risk of hospital death for burns with NI was 4.30 times higher than that for patients without it, and the cumulative probability of discharge was consistently lesser for an infected patient. Burn size was a main negative risk factor for death [18, 25-26]. The associated between TBSA and mortality was observed in the present study which was consist with the results of other studies [25,26]. Inhalation injury usually cause pulmonary and systemic complications which greatly increases the risk of death after burn and the results of our study confirmed this [25-26].

The association between NI and LOS has been illustrated by many studies. The median LOS was about 2-fold higher in trauma patients with NI compared with patients without infection [27]. Among patients with critical illness, NI increased the LOS by approximately 18 days per patients [28]. NI after burn has been considered as

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a risk factor for prolonged LOS, although clinical data are still lacking. Shupp JW et al. reported that BSI was associated with longer hospital LOS in burn patients [26]. Nevertheless, NI can only impact LOS after it has started and the duration of hospitalization prior to the infection should be controlled. So, a multistate model was used in the present study to estimation of extra LOS caused by NI. We found that the extra length of stay due to NI in burn patients was 17.68 days.

There are some limitations in the present study. First, efforts used to prevent NI, such as antibiotic treatment and surgery, may have been started before the diagnosis was made. So, our assessment of the impact of NI on LOS and hospital mortality should be regarded as a lower estimate. Second, as an observational and retrospective study, some potential factors, such as nursing protocols and the use of anti-peptic ulcer or immunosuppression drugs, that may be associated with NIs, LOS and death were not available. These factors need to be taken into consideration in the prospective studies. Additionally, the present study was performed in a single center and the results need to be further confirmed by multiple center trials.

Conclusion:

The present study provided additional information about the impact of NI on LOS and hospital mortality in burn patients. Using competing risk and multistate model, we found that nosocomial infection was associated with the increased cumulative incidence of burn death. The expected extra Length of stay due to nosocomial infections among burn patients was 14.6 days. The model used in the present study may help to improve the accuracy of estimates of LOS and incidence of death due to NIs in burns.

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Conflict of interest:

The authors declare that they have no completing interests

Authors' contributions

HLG and ZJL wrote the protocol, participated in the data analysis, and contributed to writing this manuscript. GJZ, XWL, JJX and CJL collected the data. All authors have read and approved the final manuscript.

Ethics approval: Institutional Review Board of the First Affiliated Hospital of Wenzhou Medical University.

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Figure legends

Figure.1 Multistate model. Our model including four states: admission, nosocomial infection, discharge alive and death. After admission, patients may be infected or not, then they may be discharge alive or die.

Figure.2 Characteristics of nosocomial infections. BWI: Burn wound infection; BSI: blood stream infection; PI: pulmonary infection; UTI: urinary tract infection.

Figure.3 Cumulative incidence functions for discharge (A) and death (B) in burn patients. read lines: nosocomial infection; black lines: no nosocomial infection.

Figure 4. Extra length of stay in patients without (red line) and with (black line) infection. LOS: length of stay. NI: nosocomial infection

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Table 1. Demographics and clinical characteristic of burn patients with and without nosocomial infection.

Variables	Total n=986	NI n=156	No-NI n=830	*P* value
Male, n(%)	642 (65.1%)	105 (64.7%)	537 (67.3%)	0.530
Age (years), median (25th, 75th)	37 (18,49)	37 (17,49)	37 (24,37)	0.470
≥65 years, n(%)	70 (7.1%)	20 (12.8%)	50 (6.0%)	**0.002**
Diabetes, n (%)	38 (3.9%)	11 (7.1%)	27 (3.3%)	**0.024**
TBSA, n (%)				
<10%	469 (47.6%)	55 (35.3%)	414 (49.9%)	**0.031**
10-29%	304 (30.8%)	38 (24.4%)	266 (32.0%)	0.056
≥30%	213(21.6%)	63 (40.3%)	150 (18.1%)	**<0.001**
Full thickness burn, n (%)	221 (22.4%)	60 (38.5%)	161 (19.4%)	**<0.001**
Inhalation injury, n (%)	46 (4.7%)	38 (24.3%)	8 (1.0%)	**<0.001**
Burn type, n (%)				
flame	771 (78.2%)	118 (75.6%)	653 (84.7%)	0.4
scalding	96 (9.7%)	11 (7.1%)	85 (10.2%)	0.218
electric	73 (7.4%)	15 (9.6%)	58 (7.0%)	0.25
others	46 (4.7%)	12 (7.7%)	34 (4.1%)	0.051
Length of hospital stay				
median (25th, 75th)	14 (8, 28)	27 (13.25, 57.75)	13 (7, 24)	**<0.001**

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In-hospital mortality, n (%)	54 (5.5%)	25 16.0	29 3.5	**<0.001**

NI: nosocomial infection; TBSA: total body surface area

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Table.2 Results of the Cox-proportional hazard analysis of nosocomial infection and hospital death

Outcomes	Variables	HR	CI_{95}	P value
NI	Age>65 years	1.863	1.135 ~3.060	0.014
	Full thickness burn	1.721	1.235~2.398	<0.001
	Inhalation injury	3.618	2.378~5.505	<0.001
Death	Nosocomial infection	4.301	2.229~8.298	<0.001
	TBSA	1.751	1.169~2.621	0.006
	Inhalation injury	2.740	1.436~5.227	0.002

NI: nosocomial infection; TBSA: total body surface area; HR: hazard ratio; CI_{95}: 95% confidence interval

Table.3 Results of multiple linear regressions analysis of length of stay (days)

Variables	B	CI_{95}	P value
TBSA	0.085	0.056~0.113	<0.001
Full thickness burn	0.105	0.052~0.157	<0.001
Electric burn	0.228	0.129~0.328	<0.001
Flame burn	0.093	0.031~0.155	<0.001
Nosocomial infection	0.244	0.184~0.305	<0.001

TBSA: total body surface area; CI_{95}: 95% confidence interval

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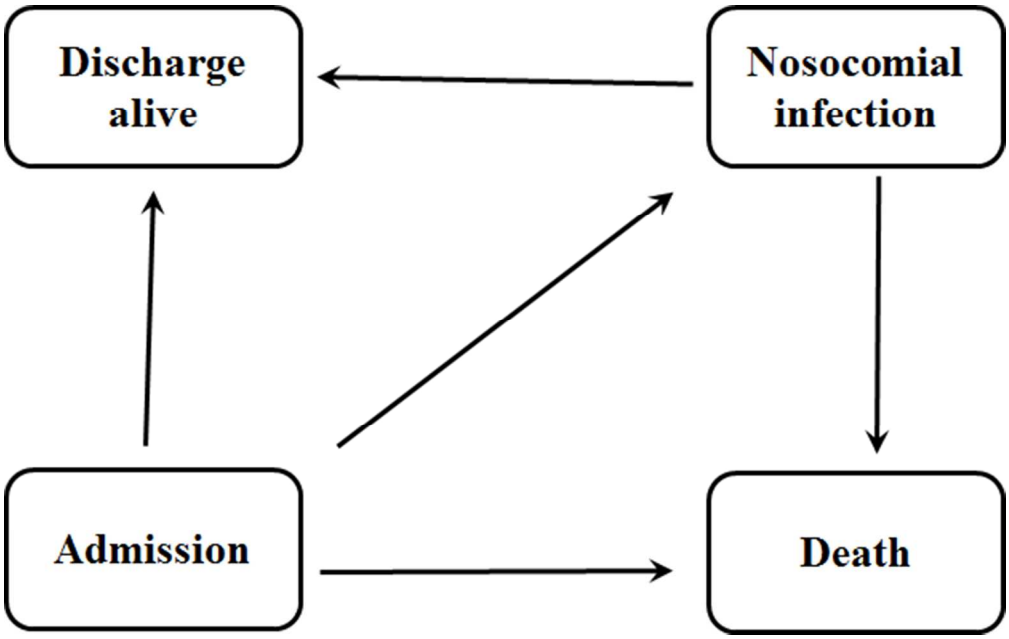

Figure.1 Multistate model.

220x138mm (300 x 300 DPI)

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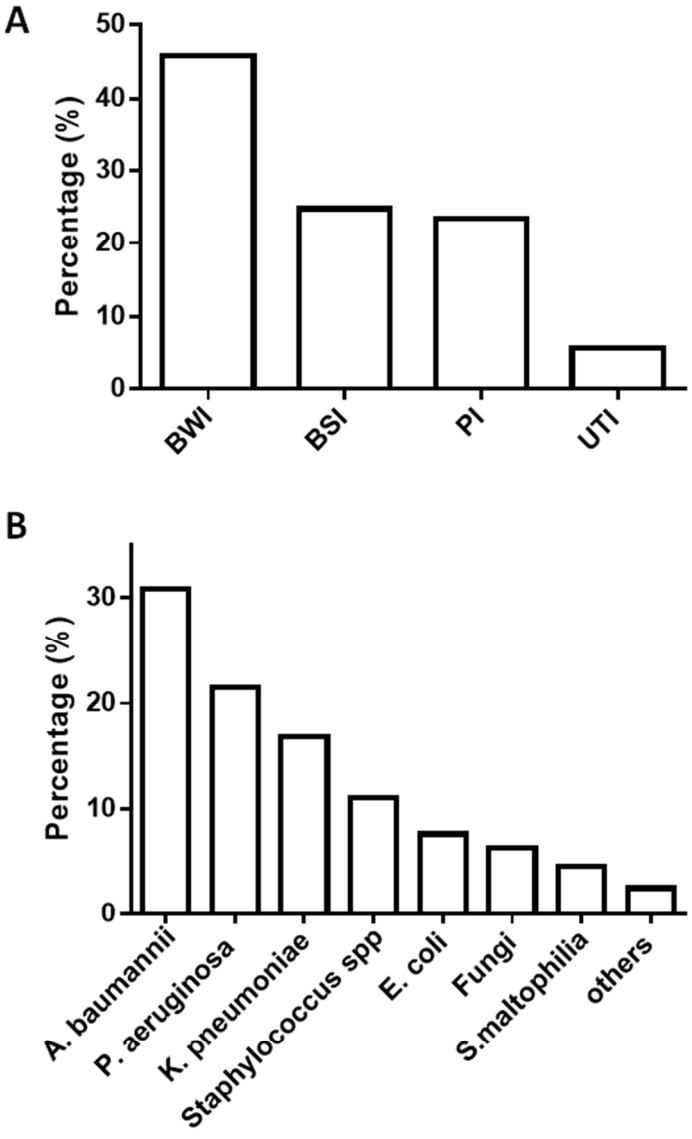

Characteristics of nosocomial infections

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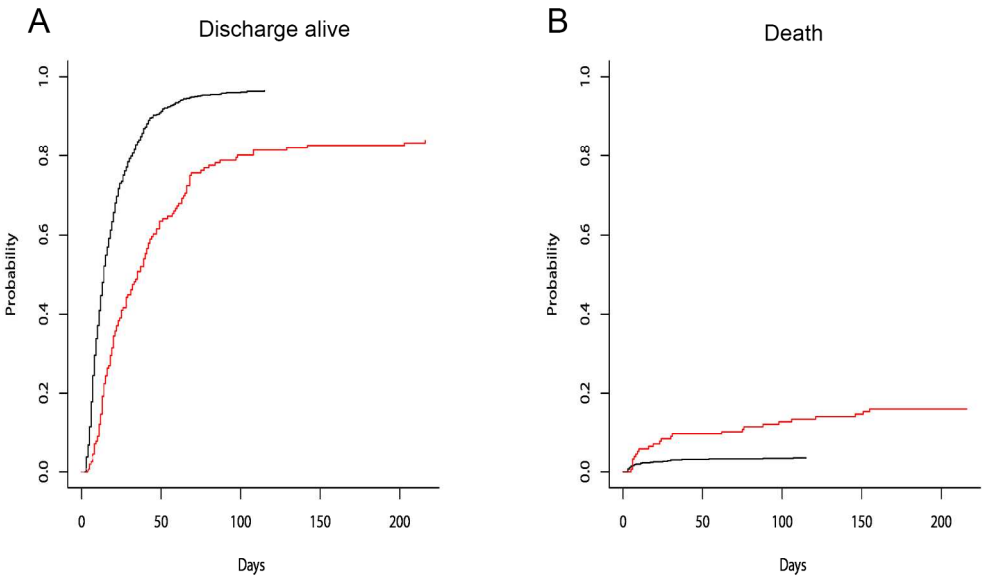

Cumulative incidence functions for discharge (A) and death (B) in burn patients

190x127mm (300 x 300 DPI)

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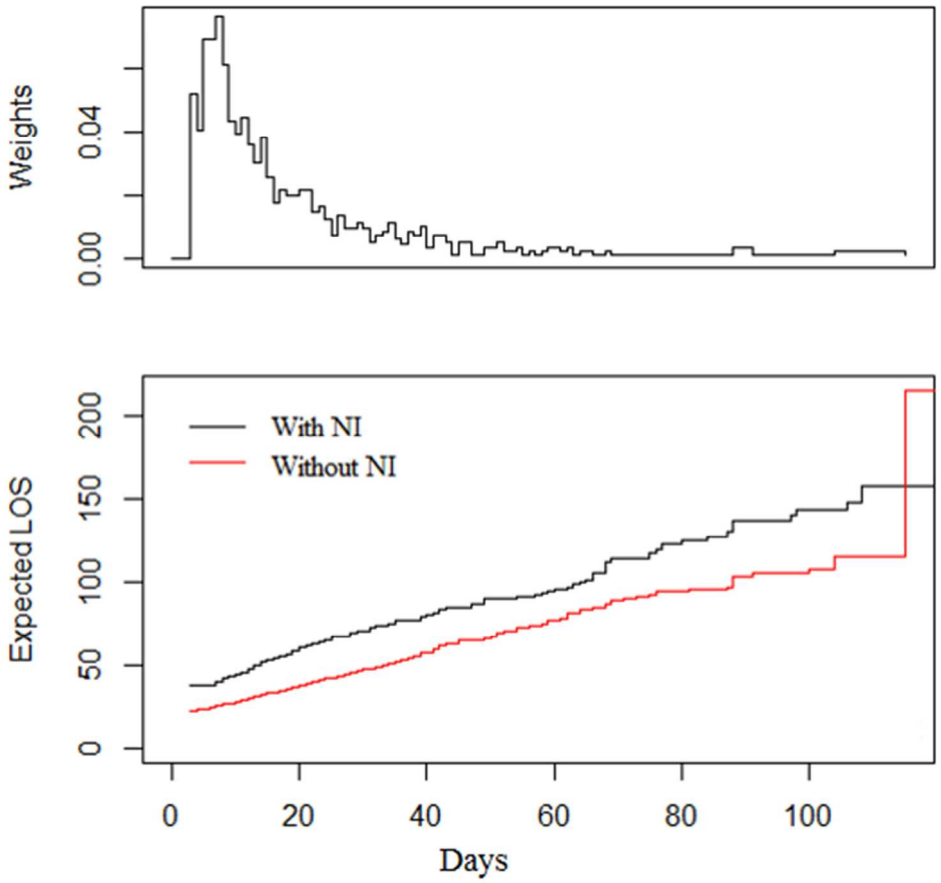

Extra length of stay in patients without (red line) and with (black line) infection.

275x275mm (300 x 300 DPI)

Table S1. Baseline characteristics of burn patients according to survive or not.

Variables	Survivors (n=932)	Non-Survivors (n=54)	*P* value
Male, n(%)	604 (64.8%)	38 (70.4%)	0.405
Age (years), median (25th, 75th)	37 (18, 49)	40 (24, 50)	0.305
≥65 years, n(%)	64 (6.9%)	6 (11.1%)	0.238
Diabetes, n (%)	36 (3.9%)	2 (3.7%)	0.953
TBSA, n (%)			
<10%	464 (49.8%)	5 (9.3%)	**<0.001**
10-29%	287 (30.8%)	17 (31.5%)	0.915
⩾30%	181 (19.4%)	32 (59.3%)	**<0.001**
Full thickness burn, n (%)	208 (22.3%)	13 (24.1%)	0.763
Inhalation injury, n (%)	24 (2.6%)	22 (40.7%)	**<0.001**
Burn types, n (%)			
Flame	727 (78.0%)	44 (81.5%)	0.547
Scalding	89 (9.5%)	5 (9.3%)	0.944
Electric	71 (7.6%)	2 (2.7%)	0.285
Others	43 (4.6%)	3 (5.6%)	0.750
NI, n (%)	131 (14.1%)	25 (46.3%)	**<0.001**

NI: nosocomial infection; TBSA: total body surface area

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STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	P2 1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	P4 2	Explain the scientific background and rationale for the investigation being reported
Objectives	P4 3	State specific objectives, including any prespecified hypotheses
Methods		
Study design	P4 4	Present key elements of study design early in the paper
Setting	P4 5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls P5 *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	P5 7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	P5-6 8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	P5-6 11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	P6-7 12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses

Continued on next page

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Results		
Participants	13*	P7 (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	P7 (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		P7 *Cross-sectional study*—Report numbers of outcome events or summary measures
Main results	P8 16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	P11 18	Summarise key results with reference to study objectives
Limitations	P3,11 19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	P3 20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	P11 22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

Zheng-jun LIU
2018/02/09

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Using competing risk and multistate model to estimate the impact of nosocomial infection on length of stay and mortality in burn patients in Southeast China

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Keywords:	burn, nosocomial infection, length of stay, mortality, multi-state model

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Using competing risk and multistate model to estimate the impact of nosocomial infection on length of stay and mortality in burn patients in Southeast China

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Abstract:

Objective: Due to the defects in skin barrier function and immune response, burn patients who survive the acute phase of a burn injury are at a high risk of nosocomial infection (NI). The aim of this study is to evaluate the impacts of NI on length of stay (LOS) and hospital mortality in burn patients using a multistate model. **Design and Setting:** A retrospective observational study was conducted in burn unit and intensive care unit (ICU) in the First Affiliated Hospital of Wenzhou Medical University, Wenzhou, China. **Participants:** Data were obtained from 1143 records of patients admitted with burn between 1 January 2013 to 31 December 2016. **Methods:** Risk factors for NIs were determined by binary logistic regression. The extended Cox model with time-varying covariates was used to determine the impact of NIs on hospital mortality, and Cumulative incidence functions (CIF) were calculated. Multiple-linear regression analysis was applied to detect the variables associated with LOS. Using a multi-state model, the extra LOS due to NI were determined. **Results:** 15.8 percent of total burn patients were suffered from NIs and incidence density of NIs was 9.6 per 1000 patient-days. NIs significantly increased the rate of death (hazard ratio: 4.625, CI95 2.361~9.062, P<0.001). The cumulative probability of death for patients with NI was greater that for those without NI. The extra LOS due to NIs was 17.68 days (CI_{95} 11.31~24.05). **Conclusions:** Using appropriate statistical methods, the present study further illustrated that NIs was associated with the increased cumulative incidence of burn death and increased LOS in burn patients.

Key words: burn, nosocomial infection, length of stay, mortality, multi-state model

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Strengths and limitations of this study

1. Using Multi-state and competing risks analysis, the present study assessed the impact of nosocomial infections on hospital mortality and length of stay in burn patients.

2. Some potential factors, such as nursing protocols and the history of immunosuppression drugs, that may be associated with NI, length of stay (LOS) and mortality were not recorded.

3. This study was performed in a single center and the results need to be further confirmed by multiple center trials.

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Introduction

Burn injury, as a common cause of morbidity and mortality, has been recognized as a global public health problem. According to the data from World Health Organization (WHO), burns account for an estimated 300, 000 deaths each year [1]. Previous evidence illustrated that burn shock and inhalation injury were the major cause of early death among patients with burn injury [2,3]. Due to the advance in fluid resuscitation, surgical approach, organ function protection, antibiotic innovation and other adjunct strategies, the early mortality of burn patients decreased dramatically over the last 30 years [4,5]. On the other hand, because of the defects in skin barrier function and immune response, burn patients who survive the acute phase of a burn injury are at a high risk of acquiring nosocomial infection (NI) [6].

It has been reported that about 30-80% of burn patients suffered from NIs [7-9]. Nevertheless, the exact impact of NIs on the LOS and mortality of burn patients remains elusive. Williams *et al* [10] investigated the predominant causes of death in burned pediatric patients. They found that infection is the leading cause of death after burn injury. A recent study reported an incidence density of 14.7 infections/1000 patient days in burn patients and NIs was not the most significant risk factors for mortality [11]. It should be noted that NI is a time-varying factor, and it can develop at any time after admission. Matched-cohort study is the most commonly used method for estimating length of stay (LOS) associated with NIs. However, different matching factors were used in different studies, and it may be difficult to identify appropriate matching factors for NIs [12]. More importantly, the time-dependent characteristics of NIs implies that infection can impact on LOS only after the infection has started [12,13]. So, appropriate statistical methods for estimating the risk of death and LOS due to NI among burn patients would be helpful in making medical decisions and developing policy. Multistate modelling is a method to avoid time-dependent bias, and it is a useful way of describing a process in which a patient moves through a

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series of states in continuous time [12,13]. The aim of this study was to determine the impacts of NI on length of stay (LOS) and hospital mortality in burn patients using a multistate model.

Materials and methods

1. Patients

A retrospective study was conducted in burn unit and intensive care unit (ICU) in the First Affiliated Hospital of Wenzhou Medical University, Wenzhou, China. The burn unit has 72 beds and there are 50 beds in the ICU. After approval by the Institutional Review Board of the First Affiliated Hospital of Wenzhou Medical University, data of total 1143 patients admitted with burn were collected during January 2013 to December 2016. Inclusion criteria: (1) age of 0-99 years; (2) admission to hospital no later than 3 days post-burn; (3) LOS>48 hours. As the present study was an observational and retrospective study, informed consent was waived by the Medical Ethics Committee.

2. Data collection

NI in burn patients was defined as infection occurring 48 hours after hospital admission. There were four main types of NIs (burn wound infection, bloodstream infection, pneumonia, urinary tract infection) according to the criteria of the Centers for Disease Control and Prevention (CDC) [14]. A case of burn wound infection was defined as a patient has change in burn wound appearance or character or patient with a burn has at least two of the following without other recognized cause: fever [Temperature (T) >38°C], hypothermia (T<36°C), hypotension, oliguria (<20 cm^2/hr), hyperglycemia at previously tolerated levels of dietary carbohydrate, or mental confusion, in which histologic examination of burn biopsy shows invasion of organisms into adjacent viable tissue. Bloodstream infection (BSI) includes laboratory-confirmed BSI and clinical sepsis. Patient with laboratory-confirmed BSI must have a recognized pathogen cultured from one or more blood cultures and organism cultured from blood is not related to an infection at another site. Clinical

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sepsis must meet the following clinical signs or symptoms with no other recognized cause: Fever (T>38°C), hypotension (systolic pressure ≤90 mm Hg), or oliguria (<20 cm3/h); blood culture not done or no organisms or antigen detected in blood; and no apparent infection at another site and physician instituted treatment for sepsis. Patients had rales or dullness to percussion on physical examination of the chest or a chest radiographic examination that showed new or progressive infiltrate or consolidation, cavitation, or pleural effusion and new onset of purulent sputum or change in character of sputum were diagnosed with pneumonia. Finally, urinary tract infection patient with the following signs or symptoms with no other recognizable cause: fever (T>38°C), urgency, frequency, dysuria, or suprapubic tenderness; and at least one of the following: 1) positive dipstick for leukocyte esterase and/or nitrate; 2) positive urine microscopy or urine culture.

Patients with a history of smoke or fire exposure in a closed space or maxillofacial burn were suspected to have inhalation injury. The diagnosis of inhalation injury was made if the suspected patients had physical findings including changes in voice and carbonaceous sputum production, or had bronchoscopic evidence [15].

The characteristics of NI including time, site and pathogen were recorded. For patients with NI at the same site, only the first episode of it was analyzed. the potential factors which are associated with NIs, LOS and mortality were collected, including gender, age, history of diabetes, date of admission, burn types (flame, scalding, electric and others), burn size and depth and inhalation injury [7-9]. Additionally, the dates of discharge and death.

3. Management

Resuscitation were performed according to the modified Evans (Ruijin) formula as described by previous paper [16,17]. Dressings were changed every 1-3 days by doctors. Silver sulfadiazine were applied on deep partial-thickness and full-thickness burns. For full-thickness burns, early surgical excision of burn eschar and biological closure were performed when the patients' condition permits. Prophylactic antibiotic

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therapy was performed in patients who needing surgical intervention (perioperative period of debridement or auto skin grafting) or requiring mechanical ventilation. The strategy of Prophylactic antibiotic therapy was mainly based on the advice of doctors from the department of microbiology and infectious diseases and the previous antibiotic susceptibility pattern of the center. Additionally, patients met CDC criteria or with identified pathogens were treated with antibiotics and adjusted according to the results of isolate's susceptibility.

4. Statistical analysis

Data are presented as a percentage of a percentage of total or interquartile ranges ($25t^{h}$ and 75^{th} percentiles), as appropriate. Mann-Whitney U test is used to analysis continuous variables while categorical variables were analyzed by the Chi-square test. Univariate analysis was performed to assess the potential variables associated with NI and hospital mortality. Variables included in the univariate analysis were age, sex, diabetes, burn types (flame, scalding, electric and others), TBSA (<10%, 10-29%, ≥30%), full thickness of burn, and inhalation injury. The variables with p-value less than 0.05 were used for further analysis. Cox model were used to determine the risk factors for NI and death. In Cox model, NI was modeled as a time-varying covariate by the 'survival' package in R. Cumulative incidence functions were calculated by the "cmprsk" package. Additionally, linear regression analysis was applied to detect the variables associated with hospital LOS. The 'etm' package in R was performed to calculate the difference in length of stay between patients with and without NI. There are four states in our multistate model: admission (state 0), NI (state 1), discharge alive (state 2) and death (state 3). After admission, patients with NIs move from state 0 into state 1, then into state 2 or state 3, while non-infected patients directly move from state 0 into state 2 or state 3. The detail information about this multistate model were shown in Figure 1. R 3.4.1 software and SPSS 18.0 were used to prepare and analysis the data. Statistical significance was expressed as both p values and 95% confidence intervals (CI_{95}). A two-sided p-value <0.05 was considered statistically significant.

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5. Patient and public involvement

No patients were involved in developing the hypothesis or research questions. No patients were involved in the development of the outcome measures. No patients were involved in developing plans for design or implementation of the study. There are no plans to disseminate the results of the research to study participants.

Results:

1. Patient characteristics

During the study period, a total of 1143 burn patients were admitted to the hospital. 157 burn patients were ineligible by exclusion criteria, and 986 patients were included in the final analysis. Demographic and burn-related characteristics are shown in Table 1. 65.1 percent of the patients were men and 34.9% were women. The median age was 37 (interquartile range [IQR], 18-49) years and 7.1% were elderly patients (65 years and older). 47.6% of the patients had < 10% TBSA burn, 30.8% had 10-29% TBSA burn, and 21.6% of burn patients with TBSA more than 30%. The main burn type is flame (78.2%), followed by scalding (9.7%), electric (7.4%) and other types (4.7%). There were 46 (4.7%) patients had inhalation injury. The hospital morality was 5.5% (54/986) and the median length of hospital stay was 14 (IQR 8-28).

2. Characteristics of NIs

156 burn patients had 209 NIs, and the median time from admission to the NI was 7 days (IQR 5-10). Over all NI rate was 9.6 per 1000 patient-days. Among all NIs, burn wound infection (BWI) was the most frequent infection (45.9%), followed by blood stream infection (BSI) (24.8%), pneumonia (23.4%) and urinary tract infection (UTI) (5.7%) (Fig.2A). As shown in Fig.2B, a total 237 microorganisms were isolated. The most common pathogens was *Acinetobacter baumannii* (30.8%), followed by *Pseudomonas aeruginosa* (21.5%), *klebsiella pneumoniae* (16.9%) and *Staphylococcus spp* (11%) (Fig.2B).

Univariate analysis indicated that there were significant differences in age, diabetes, TBSA<10%, TBSA≥30%, full thickness burn, inhalation injury and LOS

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between burn patients with and without NIs (Table 1). Using a Cox regression model, there was a statistically significant increased odds ratio for NI in patients with age (HR 1.014; CI_{95} 1.005-1.022 , P<0.01), full thickness burn (HR 1.702; CI_{95} 1.218~2.378, p<0.01) and inhalation injury (OR 3.202; CI_{95} 2.091~4.903, p<0.001) (Table 2).

3. Impact of NIs on hospital death of burn patients

As shown in Table 1, the hospital mortality of patients with and without NI were 16.0% and 3.5%, respectively. Univariate analysis indicated that the hospital mortality of patients with NIs were higher than those without NIs (Table S1). Using a Cox regression model with NI modeled as a time-varying covariate, we found the risk of hospital death for patients with NI was 5.92 times higher than that for patients without it (CI_{95} 3.098~11.31, P<0.001). After adjusting for age, gender, TBSA and inhalation injury, the risk of hospital death for patients with NI was 4.625 times higher than for patients without NI (CI_{95} 2.361~9.062, P<0.001) (Table 2, Table S1). Cumulative incidence functions for death were shown in Fig 3A. The cumulative probability of discharge was consistently lesser for an infected patient (left panel). As shown in Fig 3B, the cumulative probability of death for a patient with NI was greater that for a patient without NI (right panel).

4. Extra length of stay

As shown in Fig.1, the median LOS for patients without NI was 13 days (IQR 7-24). For patients with NI, the median LOS was 27 days (IQR 13.25-57.75). Because the LOS distribution is positively skewed, the logarithm (base 10) of LOS was used as the response variable in multiple linear regressions. Based on the results of multiple linear regressions, NI was associated with LOS in burn patients. Other variables associated with LOS were TBSA, electric burn, flame burn, full thickness (Table 3). Using a multi-state model, the extra length of stay due to NI was 17.68 days [CI_{95} 11.31~24.05, standard error (SE): 3.25, P<0.001] (Fig. 4).

Discussion:

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Burn patients are at high risk for local and systemic infections. Although infection control program has been performed in most burn centers and hospitals, the incidence of NI remains high. Alp E et al. reported 11 percent of burn patients were suffered from NI and incidence density was 14.7 per 1000 patient days [11]. Recently, a prospective cohort study was conducted in six major US burn centers to determine the association between burn size and the morbidity and mortality of burns. It found that, in patients who have > 20% TBSA burn and need for surgical intervention, the incidence of NI was 70% [18]. In the present study, incidence density of NI was 9.6 per 1000 patient days which was less than that reported by Alp E et al. and Jeschke MG et al. BWI was the most common infections in our burn center. *A. baumannii* and P. aeruginosa accounted for about 50% of total isolates, and *A. baumannii* was the predominant pathogen. Previously study illustrated that *A. baumannii* was the most common Gram-negative pathogen in burn patients [9]. According to the data published by Alp E et al [11], 57% of isolates from burns was *A. baumannii* in 2009. Nowadays, *A. baumannii* has emerged as an important pathogen causing NIs in China. Rigorous antibiotic stewardship and infection control measures were applied to prevent the spread of *A. baumannii* infections [19].

Many factors contribute to NIs in burns, including burn injury induced immunosuppression [6-9]. Clinical and experimental evidence illustrated that severe systemic inflammation after burn injuries can lead to a compensatory anti-inflammatory response, which is characterized by decreased number of T helper lymphocytes, increased suppressive activity of Foxp3+T cells as well as elevated levels of anti-inflammatory cytokines [20-22]. Declines in immune function with age make the elderly more susceptible to infections [12,23]. The present study showed that burn patients older than 65 years were more susceptible to NI. Similar to the previous reports, full thickness burn and inhalation injury were observed to be associated with NI [8,9,11].

The most notable finding in this study was the association between NIs and hospital mortality in burn patients. It has been reported that, in patients with more than 40%

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TBSA, over 70% of deaths were related to sepsis resulting from BWIs and other infection complications [6,8,9,11,24]. Nevertheless, some evidence illustrated that NIs were not the main cause of death in overall burn patients. The different severity of burn injury and statistical method may contribute to the different results. In the present study, NI was modeled as a time-varying factor in a competing risk model. The results illustrated that the risk of hospital death for burns with NI was 4.625 times higher than that for non-infected patients, and the cumulative probability of discharge was consistently lesser for an infected patient. Burn size was the strongest predictor of mortality in burns, as illustrated by previous studies [18, 25-26]. In the present study, we found that TBSA is a risk factor for hospital death in burn patients. Inhalation injury usually cause pulmonary and systemic complications which greatly increases the risk of death after burn and the results of our study confirmed this [25-26].

The association between NI and LOS has been illustrated by many studies. The median LOS was about 2-fold higher in trauma patients with NI compared with patients without infection [27]. Among patients with critical illness, NI increased the LOS by approximately 18 days per patients [28]. NI after burn has been considered as a risk factor for prolonged LOS. Shupp JW et al. reported that BSI was associated with longer hospital LOS in burn patients [26]. Nevertheless, there were no studies to assess the exact impact of NIs on LOS in burns. Additionally, the time-dependent nature of NIs implies that infection can impact on LOS only after the infection has started. While analyzing the impact of NIs on LOS, the duration of hospitalization prior to the NIs should be considered . So, a multistate model was used in the present study to estimation of extra LOS caused by NI. We found that the extra length of stay due to NI in burn patients was 17.68 days.

There are some limitations in the present study. First, efforts used to prevent NI, such as antibiotic treatment and surgery, may have been started before the diagnosis was made. So, our assessment of the impact of NI on LOS and hospital mortality should be regarded as a lower estimate. Second, as an observational and retrospective

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study, some potential factors, such as nursing protocols and the use of anti-peptic ulcer or immunosuppression drugs, that may be associated with NIs, LOS and death were not available. Additionally, factors, including mechanical ventilation and application of antibiotics, may also influence the incidence of NIs. These factors need to be taken into consideration in the prospective studies. Additionally, the present study was performed in a single center and the results need to be further confirmed by multiple center trials.

Conclusion:

The present study provided additional information about the impact of NI on LOS and hospital mortality in burn patients. Using competing risk and multistate model, we found that nosocomial infection was associated with the increased cumulative incidence of burn death. The expected extra Length of stay due to nosocomial infections among burn patients was 14.6 days. The model used in the present study may help to improve the accuracy of estimates of LOS and incidence of death due to NIs in burns.

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Conflict of interest:

The authors declare that they have no completing interests

Authors' contributions

HLG and ZJL wrote the protocol, participated in the data analysis, and contributed to writing this manuscript. GJZ, XWL, JJX and CJL collected the data. All authors have read and approved the final manuscript.

Ethics approval: Institutional Review Board of the First Affiliated Hospital of

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Figure legends

Figure.1 Multistate model. Our model including four states: admission, nosocomial infection, discharge alive and death. After admission, patients may be infected or not, then they may be discharge alive or die.

Figure.2 Characteristics of nosocomial infections. BWI: Burn wound infection; BSI: blood stream infection; PI: pulmonary infection; UTI: urinary tract infection.

Figure.3 Cumulative incidence functions for discharge (A) and death (B) in burn patients. read lines: nosocomial infection; black lines: no nosocomial infection.

Figure 4. Extra length of stay in patients without (red line) and with (black line) infection. LOS: length of stay. NI: nosocomial infection

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Table 1. Demographics and clinical characteristic of burn patients with and without nosocomial infection.

Variables	Total n=986	NI n=156	No-NI n=830	*P* value
Male, n(%)	642 (65.1%)	105 (64.7%)	537 (67.3%)	0.530
Age (years), median (25th, 75th)	37 (18,49)	37 (17,49)	37 (24,37)	0.470
Diabetes, n (%)	38 (3.9%)	11 (7.1%)	27 (3.3%)	**0.024**
TBSA, n (%)				
<10%	469 (47.6%)	55 (35.3%)	414 (49.9%)	**0.031**
10-29%	304 (30.8%)	38 (24.4%)	266 (32.0%)	0.056
≥30%	213(21.6%)	63 (40.3%)	150 (18.1%)	**<0.001**
Full thickness burn, n (%)	221 (22.4%)	60 (38.5%)	161 (19.4%)	**<0.001**
Inhalation injury, n (%)	46 (4.7%)	38 (24.3%)	8 (1.0%)	**<0.001**
Burn type, n (%)				
flame	771 (78.2%)	118 (75.6%)	653 (84.7%)	0.4
scalding	96 (9.7%)	11 (7.1%)	85 (10.2%)	0.218
electric	73 (7.4%)	15 (9.6%)	58 (7.0%)	0.25
others	46 (4.7%)	12 (7.7%)	34 (4.1%)	0.051
Length of hospital stay				
median (25th, 75th)	14 (8, 28)	27 (13.25, 57.75)	13 (7, 24)	**<0.001**
In-hospital mortality, n (%)	54 (5.5%)	25 16.0	29 3.5	**<0.001**

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NI: nosocomial infection; TBSA: total body surface area

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Table.2 Results of the Cox-proportional hazard analysis of nosocomial infection and hospital death

Outcomes	Variables	HR	CI_{95}	P value
NI	Age	1.014	1.005 ~1.022	<0.01
	Full thickness burn	1.702	1.218~2.378	<0.01
	Inhalation injury	3.202	2.091~4.903	<0.001
Death	Nosocomial infection	4.625	2.361~9.062	<0.001
	TBSA	1.459	1.089~1.955	<0.05
	Inhalation injury	2.986	1.511~5.901	<0.05

NI: nosocomial infection; TBSA: total body surface area; HR: hazard ratio; CI_{95}: 95% confidence interval

Table.3 Results of multiple linear regressions analysis of length of stay (days)

Variables	B	CI_{95}	P value
TBSA	0.085	0.056~0.113	<0.001
Full thickness burn	0.105	0.052~0.157	<0.001
Electric burn	0.228	0.129~0.328	<0.001
Flame burn	0.093	0.031~0.155	<0.001
Nosocomial infection	0.244	0.184~0.305	<0.001

TBSA: total body surface area; CI_{95}: 95% confidence interval

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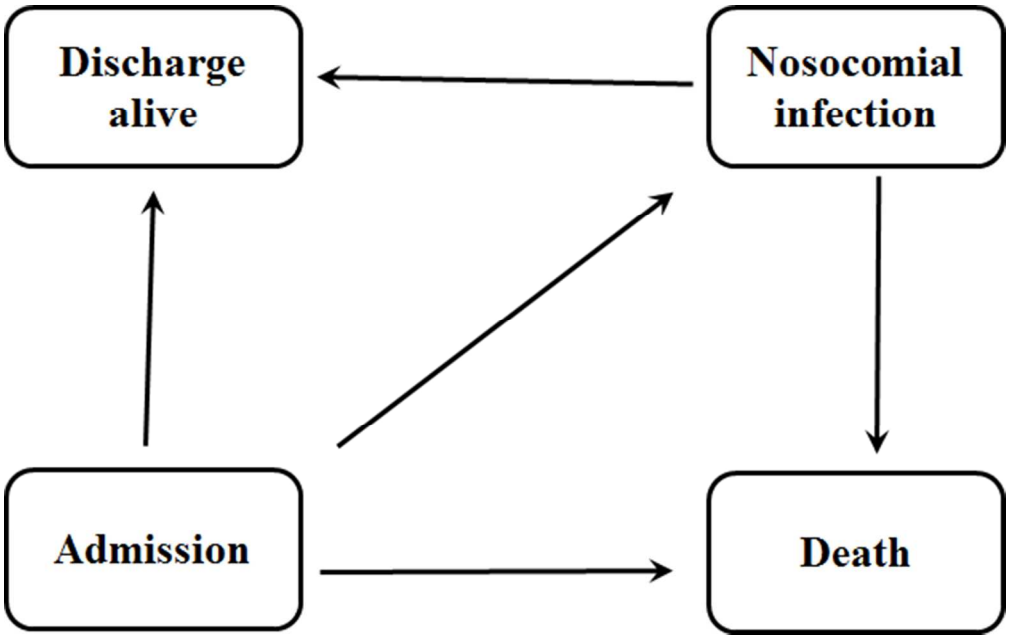

Figure.1 Multistate model.

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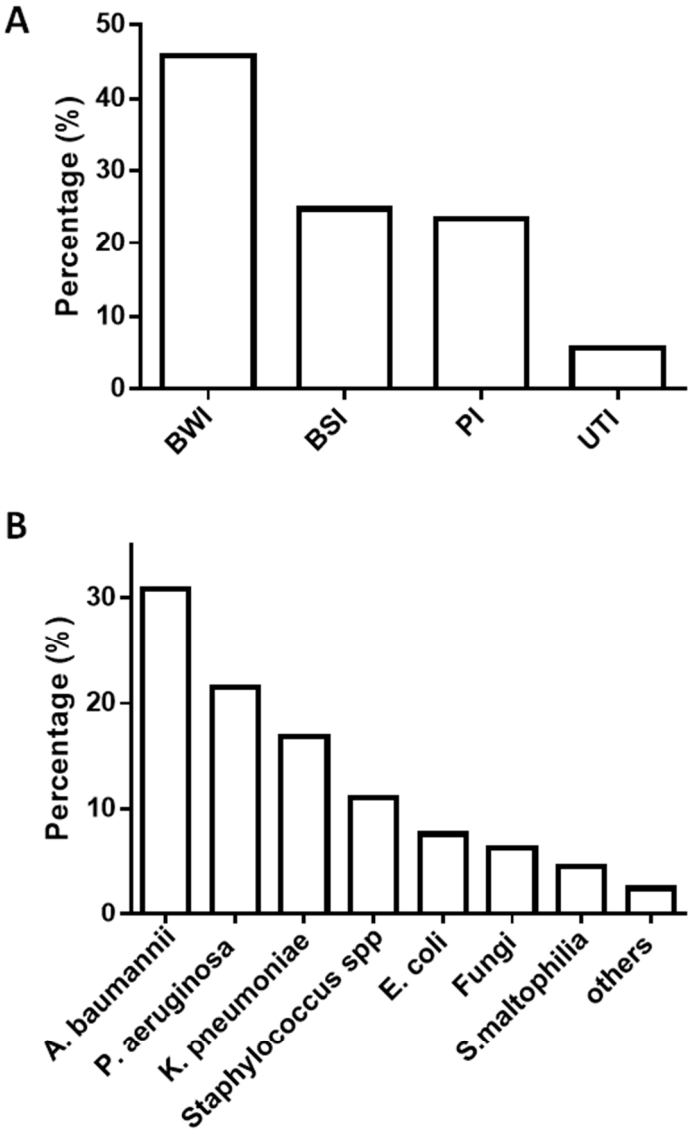

Characteristics of nosocomial infections

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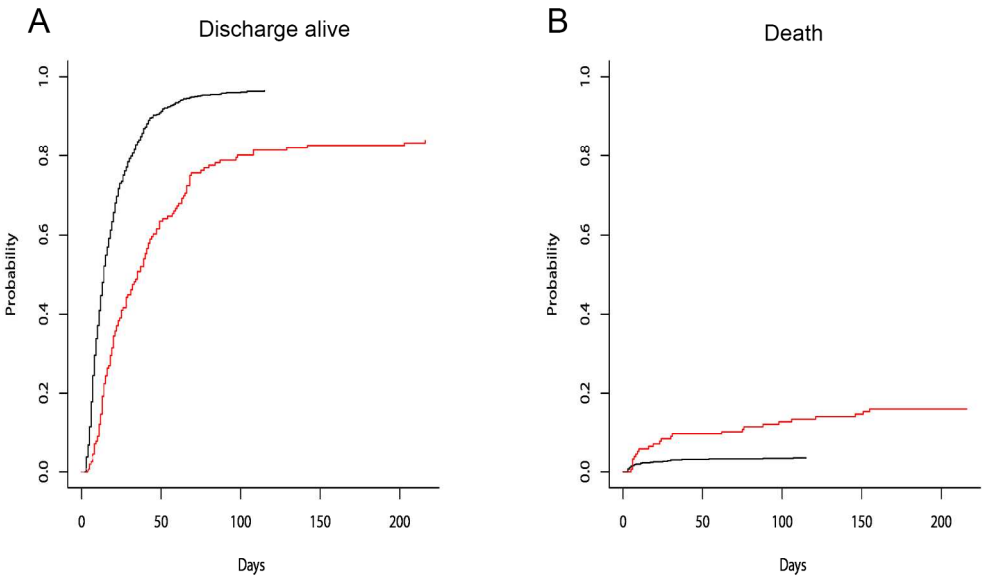

Cumulative incidence functions for discharge (A) and death (B) in burn patients

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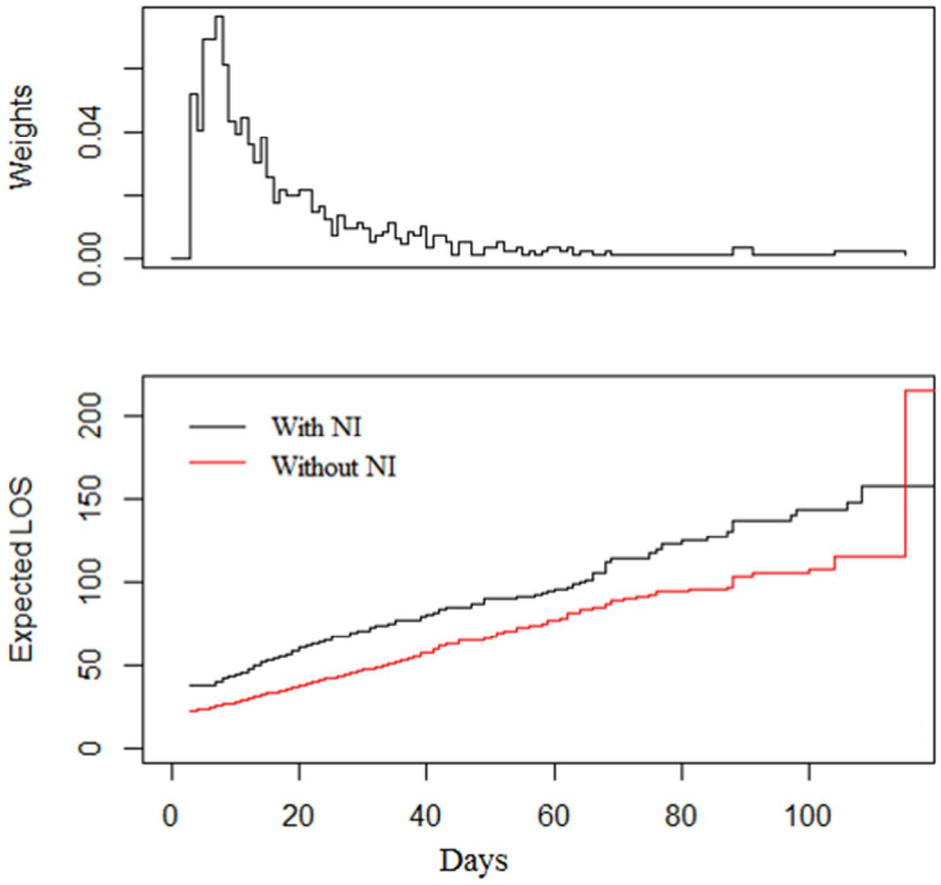
view only



Extra length of stay in patients without (red line) and with (black line) infection.

275x275mm (300 x 300 DPI)

Table S1. Baseline characteristics of burn patients according to survive or not.

Variables	Survivors (n=932)	Non-Survivors (n=54)	*P* value
Male, n(%)	604 (64.8%)	38 (70.4%)	0.405
Age (years), median (25th, 75th)	37 (18, 49)	40 (24, 50)	0.305
Diabetes, n (%)	36 (3.9%)	2 (3.7%)	0.953
TBSA, n (%)			
<10%	464 (49.8%)	5 (9.3%)	**<0.001**
10-29%	287 (30.8%)	17 (31.5%)	0.915
⩾30%	181 (19.4%)	32 (59.3%)	**<0.001**
Full thickness burn, n (%)	208 (22.3%)	13 (24.1%)	0.763
Inhalation injury, n (%)	24 (2.6%)	22 (40.7%)	**<0.001**
Burn types, n (%)			
Flame	727 (78.0%)	44 (81.5%)	0.547
Scalding	89 (9.5%)	5 (9.3%)	0.944
Electric	71 (7.6%)	2 (2.7%)	0.285
Others	43 (4.6%)	3 (5.6%)	0.750
NI, n (%)	131 (14.1%)	25 (46.3%)	**<0.001**

NI: nosocomial infection; TBSA: total body surface area

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STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	P2 1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	P4 2	Explain the scientific background and rationale for the investigation being reported
Objectives	P4 3	State specific objectives, including any prespecified hypotheses
Methods		
Study design	P4 4	Present key elements of study design early in the paper
Setting	P4 5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls P5 *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	P5 7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	P5-6 8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	P5-6 11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	P6-7 12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses

Continued on next page

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Results		
Participants	13*	P7 (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	P7 (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		P7 *Cross-sectional study*—Report numbers of outcome events or summary measures
Main results	P816	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	P11 18	Summarise key results with reference to study objectives
Limitations	P3,11 19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	P3 20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	P11 22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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2018/02/09

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Using competing risk and multistate model to estimate the impact of nosocomial infection on length of stay and mortality in burn patients in Southeast China

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Using competing risk and multistate model to estimate the impact of nosocomial infection on length of stay and mortality in burn patients in Southeast China

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Abstract:

Objective: Due to the defects in skin barrier function and immune response, burn patients who survive the acute phase of a burn injury are at a high risk of nosocomial infection (NI). The aim of this study is to evaluate the impacts of NI on length of stay (LOS) and hospital mortality in burn patients using a multistate model. **Design and Setting:** A retrospective observational study was conducted in burn unit and intensive care unit (ICU) in the First Affiliated Hospital of Wenzhou Medical University, Wenzhou, China. **Participants:** Data were obtained from 1143 records of patients admitted with burn between 1 January 2013 to 31 December 2016. **Methods:** Risk factors for NIs were determined by binary logistic regression. The extended Cox model with time-varying covariates was used to determine the impact of NIs on hospital mortality, and Cumulative incidence functions (CIF) were calculated. Multiple-linear regression analysis was applied to detect the variables associated with LOS. Using a multi-state model, the extra LOS due to NI were determined. **Results:** 15.8 percent of total burn patients suffered from NIs and incidence density of NIs was 9.6 per 1000 patient-days. NIs significantly increased the rate of death (hazard ratio: 4.266, CI_{95} 2.218~8.208, P=0.000). The cumulative probability of death for patients with NI was greater that for those without NI. The extra LOS due to NIs was 17.68 days (CI_{95} 11.31~24.05). **Conclusions:** Using appropriate statistical methods, the present study further illustrated that NIs was associated with the increased cumulative incidence of burn death and increased LOS in burn patients.

Key words: burn, nosocomial infection, length of stay, mortality, multi-state model

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Strengths and limitations of this study

1. Using Multi-state and competing risks analysis, the present study assessed the impact of nosocomial infections on hospital mortality and length of stay in burn patients.

2. Some potential factors, such as nursing protocols and the history of immunosuppression drugs, that may be associated with NI, length of stay (LOS) and mortality were not recorded.

3. This study was performed in a single center and the results need to be further confirmed by multiple center trials.

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Introduction

Burn injury, as a common cause of morbidity and mortality, has been recognized as a global public health problem. According to the data from World Health Organization (WHO), burns account for an estimated 300, 000 deaths each year [1]. Previous evidence illustrated that burn shock and inhalation injury were the major cause of early death among patients with burn injury [2,3]. Due to the advance in fluid resuscitation, surgical approach, organ function protection, antibiotic innovation and other adjunct strategies, the early mortality of burn patients decreased dramatically over the last 30 years [4,5]. On the other hand, because of the defects in skin barrier function and immune response, burn patients who survive the acute phase of a burn injury are at a high risk of acquiring nosocomial infection (NI) [6].

It has been reported that about 30-80% of burn patients suffered from NIs [7-9]. Nevertheless, the exact impact of NIs on the LOS and mortality of burn patients remains elusive. Williams *et al* [10] investigated the predominant causes of death in burned pediatric patients. They found that infection is the leading cause of death after burn injury. A recent study reported an incidence density of 14.7 infections/1000 patient days in burn patients [11]. Nevertheless, the study illustrated that NIs was not a risk factor for mortality, using logistic regression, after adjusting for confound variables [11]. It should be noted that NI is a time-varying factor, and it can develop at any time after admission. Matched-cohort study is the most commonly used method for estimating length of stay (LOS) associated with NIs. However, different matching factors were used in different studies, and it may be difficult to identify appropriate matching factors for NIs [12]. More importantly, the time-dependent characteristics of NIs implies that infection can impact on LOS only after the infection has started [12,13]. So, appropriate statistical methods for estimating the risk of death and LOS due to NI among burn patients would be helpful in making medical decisions and developing policy. Multistate modelling is a method to avoid time-dependent bias,

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and it is a useful way of describing a process in which a patient moves through a series of states in continuous time [12,13]. The aim of this study was to determine the impacts of NI on length of stay (LOS) and hospital mortality in burn patients using a multistate model.

Materials and methods

1. Patients

A retrospective study was conducted in burn unit and intensive care unit (ICU) in the First Affiliated Hospital of Wenzhou Medical University, Wenzhou, China. The burn unit has 72 beds and there are 50 beds in the ICU. After approval by the Institutional Review Board of the First Affiliated Hospital of Wenzhou Medical University, data of total 1143 patients admitted with burn were collected during January 2013 to December 2016. Inclusion criteria: (1) age of 0-99 years; (2) admission to hospital no later than 3 days post-burn; (3) LOS>48 hours. As the present study was an observational and retrospective study, informed consent was waived by the Medical Ethics Committee.

2. Data collection

NI in burn patients was defined as infection occurring 48 hours after hospital admission. There were four main types of NIs (burn wound infection, bloodstream infection, pneumonia, urinary tract infection) according to the criteria of the Centers for Disease Control and Prevention (CDC) [14]. Briefly, burn wound infection (BWI) was defined as patient has a change in burn wound appearance, such as rapid eschar separation; dark brown, black, or violaceous discoloration of escar, and at least one of the following: histologic examination of burn biopsy shows invasion of organisms into adjacent viable tissue or positive blood culture without other identifiable infection. Bloodstream infection (BSI) includes laboratory-confirmed BSI and clinical sepsis. Patient with laboratory-confirmed BSI must have a recognized pathogen cultured from one or more blood cultures and organism cultured from blood is not related to an infection at another site. Clinical sepsis must meet the following clinical

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signs or symptoms with no other recognized cause: Fever (T>38°C), hypotension (systolic pressure ≤90 mm Hg), or oliguria (<20 cm3/h); blood culture not done or no organisms or antigen detected in blood; and no apparent infection at another site and physician instituted treatment for sepsis. Patients had rales or dullness to percussion on physical examination of the chest or a chest radiographic examination that showed new or progressive infiltrate or consolidation, cavitation, or pleural effusion and new onset of purulent sputum or change in character of sputum were diagnosed with pneumonia. Finally, urinary tract infection patient with the following signs or symptoms with no other recognizable cause: fever (T>38°C), urgency, frequency, dysuria, or suprapubic tenderness; and at least one of the following: 1) positive dipstick for leukocyte esterase and/or nitrate; 2) positive urine microscopy or urine culture.

Patients with a history of smoke or fire exposure in a closed space or maxillofacial burn were suspected to have inhalation injury. The diagnosis of inhalation injury was made if the suspected patients had physical findings including changes in voice and carbonaceous sputum production, or had bronchoscopic evidence [15].

The characteristics of NI including time, site and pathogen were recorded. For patients with NI at the same site, only the first episode of it was analyzed. the potential factors which are associated with NIs, LOS and mortality were collected, including gender, age, history of diabetes, date of admission, burn types (flame, scalding, electric and others), burn size and depth and inhalation injury [7-9]. Additionally, the dates of discharge and death.

3. Management

Resuscitation were performed according to the modified Evans (Ruijin) formula as described by previous paper [16,17]. Dressings were changed every 1-3 days by doctors. Silver sulfadiazine were applied on deep partial-thickness and full-thickness burns. For full-thickness burns, early surgical excision of burn eschar and biological closure were performed when the patients' condition permits. Prophylactic antibiotic therapy was performed in patients who needing surgical intervention (perioperative

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period of debridement or auto skin grafting) or requiring mechanical ventilation. The strategy of Prophylactic antibiotic therapy was mainly based on the advice of doctors from the department of microbiology and infectious diseases and the previous antibiotic susceptibility pattern of the center. Additionally, patients met CDC criteria were treated with antibiotics. When a pathogen was identified, antibiotics were adjusted according to the results of isolate's susceptibility. The duration of antibiotics therapy is decided by the treating physician based on clinical symptoms, blood culture results as well as other infection markers.

4. Statistical analysis

Data are presented as a percentage of a percentage of total or interquartile ranges ($25t^h$ and 75^{th} percentiles), as appropriate. Mann-Whitney U test is used to analysis continuous variables while categorical variables were analyzed by the Chi-square test. Univariate analysis was performed to assess the potential variables associated with NI and hospital mortality. Variables included in the univariate analysis were age, sex, diabetes, burn types (flame, scalding, electric and others), TBSA (<10%, 10-29%, ≥30%), full thickness of burn, and inhalation injury. The variables with p-value less than 0.05 were used for further analysis. Cox model were used to determine the risk factors for NI and death. In Cox model, NI was modeled as a time-varying covariate by the 'survival' package in R. Cumulative incidence functions were calculated by the "cmprsk" package. Additionally, linear regression analysis was applied to detect the variables associated with hospital LOS. The 'etm' package in R was performed to calculate the difference in length of stay between patients with and without NI. The code used in the present study was available at https://CRAN.R-project.org/. There are four states in our multistate model: admission (state 0), NI (state 1), discharge alive (state 2) and death (state 3). After admission, patients with NIs move from state 0 into state 1, then into state 2 or state 3, while non-infected patients directly move from state 0 into state 2 or state 3. The detail information about this multistate model were shown in Figure 1. R 3.4.1 software and SPSS 18.0 were used to prepare and analysis the data. Statistical significance was expressed as both p values and 95% confidence intervals (CI_{95}). A two-sided p-value <0.05 was considered statistically

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significant.

5. Patient and public involvement

No patients were involved in developing the hypothesis or research questions. No patients were involved in the development of the outcome measures. No patients were involved in developing plans for design or implementation of the study. There are no plans to disseminate the results of the research to study participants.

Results:

1. Patient characteristics

During the study period, a total of 1143 burn patients were admitted to the hospital. 157 burn patients were ineligible by exclusion criteria, and 986 patients were included in the final analysis. Demographic and burn-related characteristics are shown in Table 1. 65.1 percent of the patients were men and 34.9% were women. The median age was 37 (interquartile range [IQR], 18-49) years and 7.1% were elderly patients (65 years and older). 47.6% of the patients had < 10% TBSA burn, 30.8% had 10-29% TBSA burn, and 21.6% of burn patients with TBSA more than 30%. The main burn type is flame (78.2%), followed by scalding (9.7%), electric (7.4%) and other types (4.7%). There were 46 (4.7%) patients had inhalation injury. The hospital morality was 5.5% (54/986) and the median length of hospital stay was 14 (IQR 8-28).

2. Characteristics of NIs

156 burn patients had 209 NIs, and the median time from admission to the NI was 7 days (IQR 5-10). Over all NI rate was 9.6 per 1000 patient-days. Among all NIs, burn wound infection (BWI) was the most frequent infection (45.9%), followed by blood stream infection (BSI) (24.8%), pneumonia (23.4%) and urinary tract infection (UTI) (5.7%) (Fig.2A). As shown in Fig.2B, a total 237 microorganisms were isolated. The most common pathogens was *Acinetobacter baumannii* (30.8%), followed by *Pseudomonas aeruginosa* (21.5%), *klebsiella pneumoniae* (16.9%) and *Staphylococcus spp* (11%) (Fig.2B).

Univariate analysis indicated that there were significant differences indiabetes, TBSA<10%, TBSA≥30%, full thickness burn, inhalation injury and LOS between

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burn patients with and without NIs (Table 1). Using a Cox regression model, there was a statistically significant increased odds ratio for NI in patients with full thickness burn (HR 1.799; CI_{95} 1.288~2.511, P=0.000) and inhalation injury (OR 3.326; CI_{95} 2.169~5.102, p=0.000), TBSA (HR1.189; CI_{95} 1.005~1.407, P=0.043) (Table 2).

3. Impact of NIs on hospital death of burn patients

As shown in Table 1, the hospital mortality of patients with and without NI were 16.0% and 3.5%, respectively. Univariate analysis indicated that the hospital mortality of patients with NIs were higher than those without NIs (Table S1). Using a Cox regression model with NI modeled as a time-varying covariate, we found the risk of hospital death for patients with NI was 5.92 times higher than that for patients without it (CI_{95} 3.098~11.310, P<0.001). After adjusting for age, gender, TBSA and inhalation injury, the risk of hospital death for patients with NI was 4.266 times higher than for patients without NI (CI_{95} 2.218~8.208, P=0.000) (Table 3, Table S1). Cumulative incidence functions for death were shown in Fig 3A. The cumulative probability of discharge was consistently lesser for an infected patient (left panel). As shown in Fig 3B, the cumulative probability of death for a patient with NI was greater that for a patient without NI (right panel).

4. Extra length of stay

As shown in Fig.1, the median LOS for patients without NI was 13 days (IQR 7-24). For patients with NI, the median LOS was 27 days (IQR 13.25-57.75). Because the LOS distribution is positively skewed, the logarithm (base 10) of LOS was used as the response variable in multiple linear regressions. Based on the results of multiple linear regressions, NI was associated with increased LOS in burn patients. Other variables associated with LOS were TBSA, electric burn, flame burn, full thickness (Table 4). Using a multi-state model, the extra length of stay due to NI was 17.68 days [CI_{95} 11.31~24.05, standard error (SE): 3.25, P<0.001] (Fig. 4).

Discussion:

Burn patients are at high risk for local and systemic infections. Although infection control program has been performed in most burn centers and hospitals, the incidence

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of NI remains high. Alp E et al. reported 11 percent of burn patients were suffered from NI and incidence density was 14.7 per 1000 patient days [11]. Recently, a prospective cohort study was conducted in six major US burn centers to determine the association between burn size and the morbidity and mortality of burns. It found that, in patients who have > 20% TBSA burn and need for surgical intervention, the incidence of NI was 70% [18]. In the present study, incidence density of NI was 9.6 per 1000 patient days which was less than that reported by Alp E et al. and Jeschke MG et al. BWI was the most common infections in our burn center. *A. baumannii* and P. aeruginosa accounted for about 50% of total isolates, and *A. baumannii* was the predominant pathogen. Previously study illustrated that *A. baumannii* was the most common Gram-negative pathogen in burn patients [9]. According to the data published by Alp E et al [11], 57% of isolates from burns was *A. baumannii* in 2009. Nowadays, *A. baumannii* has emerged as an important pathogen causing NIs in China. Rigorous antibiotic stewardship and infection control measures were applied to prevent the spread of *A. baumannii* infections [19].

Many factors contribute to NIs in burns, including burn injury induced immunosuppression [6-9]. Clinical and experimental evidence illustrated that severe systemic inflammation after burn injuries can lead to a compensatory anti-inflammatory response, which is characterized by decreased number of T helper lymphocytes, increased suppressive activity of regulatory T cells (Tregs) which specialized for immune suppression, as well as elevated levels of anti-inflammatory cytokines [20-22]. Inhibition of Tregs attenuates postburn sepsis has been confirmed by an experimental study [23]. . In addition, we also observed that full thickness burn, TBSA, and inhalation injury are the risk factors for NIs in burn patients. The most notable finding in this study was the association between NIs and hospital mortality in burn patients. It has been reported that, in patients with more than 40% TBSA, over 70% of deaths were related to sepsis resulting from BWIs and other infection complications [6,8,9,11,24]. Nevertheless, a study illustrated that NI was a risk factor for mortality in univariate analysis, but it was not found as a risk factor for mortality in the stepwise forward logistic regression undertaken to control effect of confound

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variables [11]. The different statistical method may contribute to the different results. In the present study, NI was modeled as a time-varying factor in a competing risk model. The results illustrated that the risk of hospital death for burns with NI was 4.266 times higher than that for non-infected patients, and the cumulative probability of discharge was consistently lesser for an infected patient. Burn size was the strongest predictor of mortality in burns, as illustrated by previous studies [18, 25-26]. In the present study, we found that TBSA is a risk factor for hospital death in burn patients. Additionally, inhalation injury usually causes pulmonary and systemic complications which greatly increases the risk of death after burn and the results of our study confirmed this [25-26].

The association between NI and LOS has been illustrated by many studies. The median LOS was about 2-fold higher in trauma patients with NI compared with patients without infection [27]. Among patients with critical illness, NI increased the LOS by approximately 18 days per patients [28]. NI after burn has been considered as a risk factor for prolonged LOS. Shupp JW et al. reported that BSI was associated with longer hospital LOS in burn patients [26]. Nevertheless, there were no studies to assess the exact impact of NIs on LOS in burns. Additionally, the time-dependent nature of NIs implies that infection can impact on LOS only after the infection has started. While analyzing the impact of NIs on LOS, the duration of hospitalization prior to the NIs should be considered. So, a multistate model was used in the present study to estimation of extra LOS caused by NI. We found that the extra length of stay due to NI in burn patients was 17.68 days.

There are some limitations in the present study. Initially, efforts used to prevent NI, such as antibiotic treatment and surgery, may have been started before the diagnosis was made. So, our assessment of the impact of NI on LOS and hospital mortality should be regarded as a lower estimate. Second, as an observational and retrospective study, some potential factors, such as nursing protocols and the use of anti-peptic ulcer or immunosuppression drugs, that may be associated with NIs, LOS and death were not available. Furthermore, factors, including mechanical ventilation and application of antibiotics, may also influence the incidence of NIs. These factors need

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to be taken into consideration in the prospective studies. Additionally, the present study was performed in a single center and the results need to be further confirmed by multiple center trials. Finally, there were no patients and public involved in this retrospective observational study. As patients and public involvement (PPI) is important for a clinical research [29], it needs to be done in the future studies.

Conclusion:

The present study provided additional information about the impact of NI on LOS and hospital mortality in burn patients. Using competing risk and multistate model, we found that nosocomial infection was associated with the increased cumulative incidence of burn death. The expected extra Length of stay due to nosocomial infections among burn patients was 17.68 days. The model used in the present study may help to improve the accuracy of estimates of LOS and incidence of death due to NIs in burns.

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Conflict of interest:

The authors declare that they have no completing interests

Authors' contributions

HLG and ZJL wrote the protocol, participated in the data analysis, and contributed to writing this manuscript. GJZ, XWL, JJX and CJL collected the data. All authors have read and approved the final manuscript.

Ethics approval: Institutional Review Board of the First Affiliated Hospital of Wenzhou Medical University.

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Data sharing statement: No additional data are available.

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Figure legends

Figure.1 Multistate model. Our model including four states: admission, nosocomial infection, discharge alive and death. After admission, patients may be infected or not, then they may be discharge alive or die.

Figure.2 Characteristics of nosocomial infections. BWI: Burn wound infection; BSI: blood stream infection; PI: pulmonary infection; UTI: urinary tract infection.

Figure.3 Cumulative incidence functions for discharge (A) and death (B) in burn patients. read lines: nosocomial infection; black lines: no nosocomial infection.

Figure 4. Extra length of stay in patients without (red line) and with (black line) infection. LOS: length of stay. NI: nosocomial infection

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Table 1. Demographics and clinical characteristic of burn patients with and without nosocomial infection.

Variables	Total n=986	NI n=156	No-NI n=830	*P* value
Male, n(%)	642 (65.1%)	105 (64.7%)	537 (67.3%)	0.530
Age (years), median (25th, 75th)	37 (18,49)	37 (17,49)	37 (24,37)	0.470
Diabetes, n (%)	38 (3.9%)	11 (7.1%)	27 (3.3%)	**0.024**
TBSA, n (%)				
<10%	469 (47.6%)	55 (35.3%)	414 (49.9%)	**0.031**
10-29%	304 (30.8%)	38 (24.4%)	266 (32.0%)	0.056
≥30%	213(21.6%)	63 (40.3%)	150 (18.1%)	**0.000**
Full thickness burn, n (%)	221 (22.4%)	60 (38.5%)	161 (19.4%)	**0.000**
Inhalation injury, n (%)	46 (4.7%)	38 (24.3%)	8 (1.0%)	**0.000**
Burn type, n (%)				
flame	771 (78.2%)	118 (75.6%)	653 (84.7%)	0.400
scalding	96 (9.7%)	11 (7.1%)	85 (10.2%)	0.218
electric	73 (7.4%)	15 (9.6%)	58 (7.0%)	0.250
others	46 (4.7%)	12 (7.7%)	34 (4.1%)	0.051
Length of hospital stay				
median (25th, 75th)	14 (8, 28)	27 (13.25, 57.75)	13 (7, 24)	**0.000**
In-hospital mortality, n (%)	54 (5.5%)	25 16.0	29 3.5	**0.000**

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NI: nosocomial infection; TBSA: total body surface area

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Table.2 Results of the Cox-proportional hazard analysis of nosocomial infection

Variables	HR	CI_{95}	P value
LOS	1.002	0.996~1.007	0.547
TBSA	1.189	1.005~1.407	**0.043**
Full thickness burn	1.799	1.289~2.511	**0.000**
Inhalation injury	3.326	2.169~5.102	**0.000**
Diabetes	1.586	0.856~2.939	0.143

LOS: length of hospital stay; TBSA: total body surface area; HR: hazard ratio; CI_{95}: 95% confidence interval

Table.3 Results of the Cox-proportional hazard analysis of hospital death

Variables	HR	CI_{95}	P value
Nosocomial infection	4.266	2.218~8.208	**0.000**
TBSA	1.374	1.034~1.825	**0.028**
Inhalation injury	2.824	1.448~5.508	**0.002**
age	1.003	0.991~1.016	0.608
gender	1.212	0.667~2.201	0.528

TBSA: total body surface area; HR: hazard ratio; CI_{95}: 95% confidence interval

Table.4 Results of multiple linear regressions analysis of length of stay (days)

Variables	B	CI_{95}	P value
TBSA	0.085	0.056~0.113	0.000
Full thickness burn	0.105	0.052~0.157	0.000
Electric burn	0.228	0.129~0.328	0.000
Flame burn	0.093	0.031~0.155	0.003
Nosocomial infection	0.244	0.184~0.305	0.000

TBSA: total body surface area; CI_{95}: 95% confidence interval

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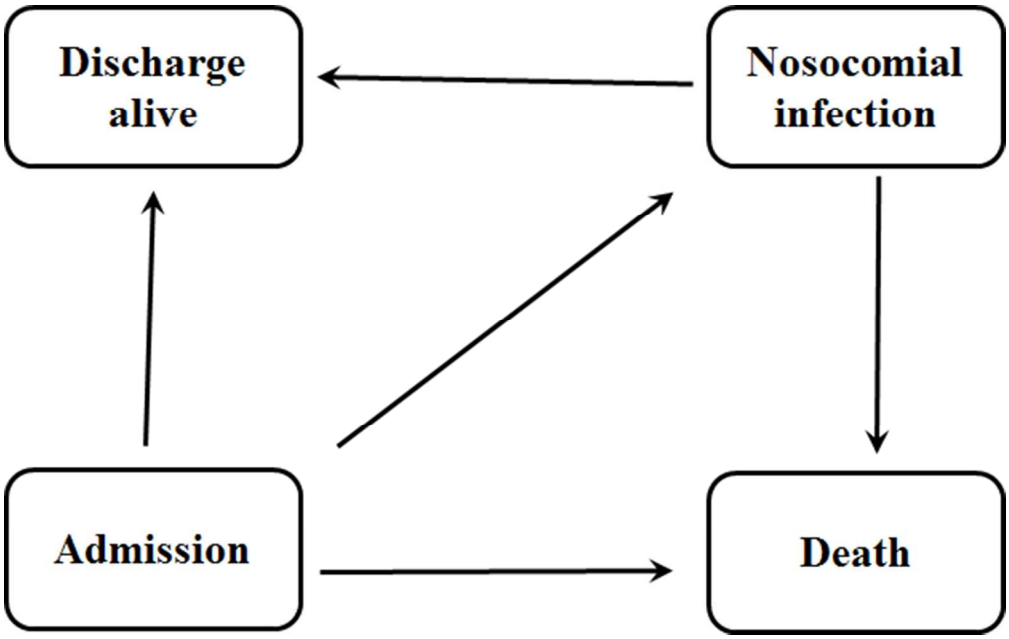

Figure.1 Multistate model.

220x138mm (300 x 300 DPI)

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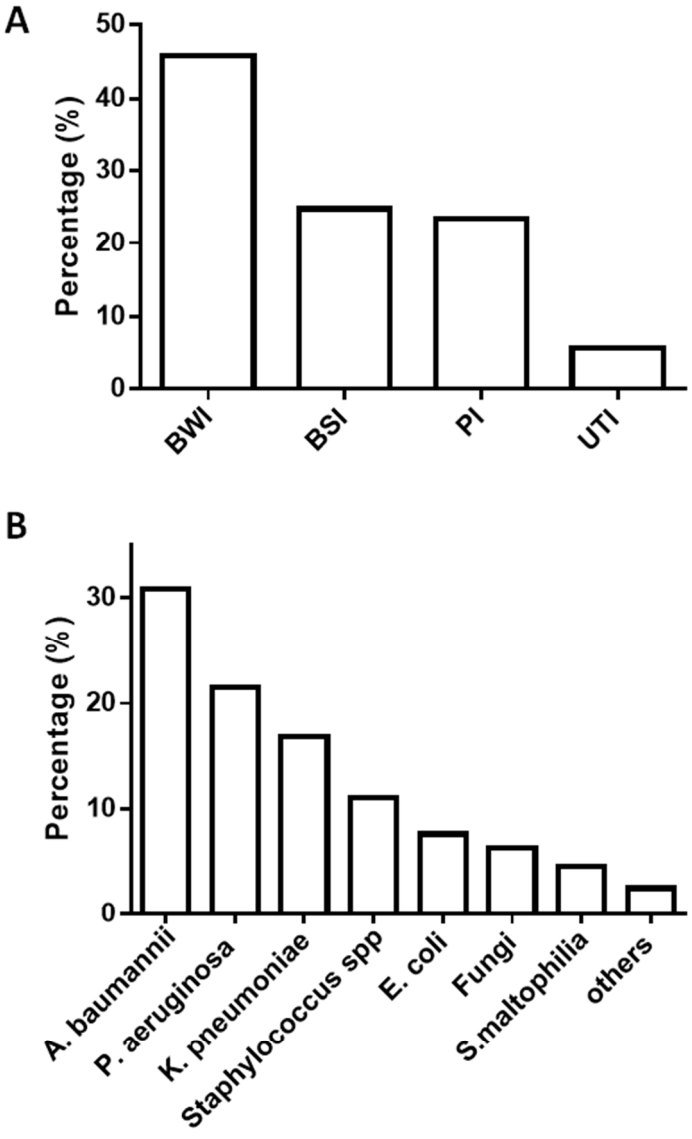
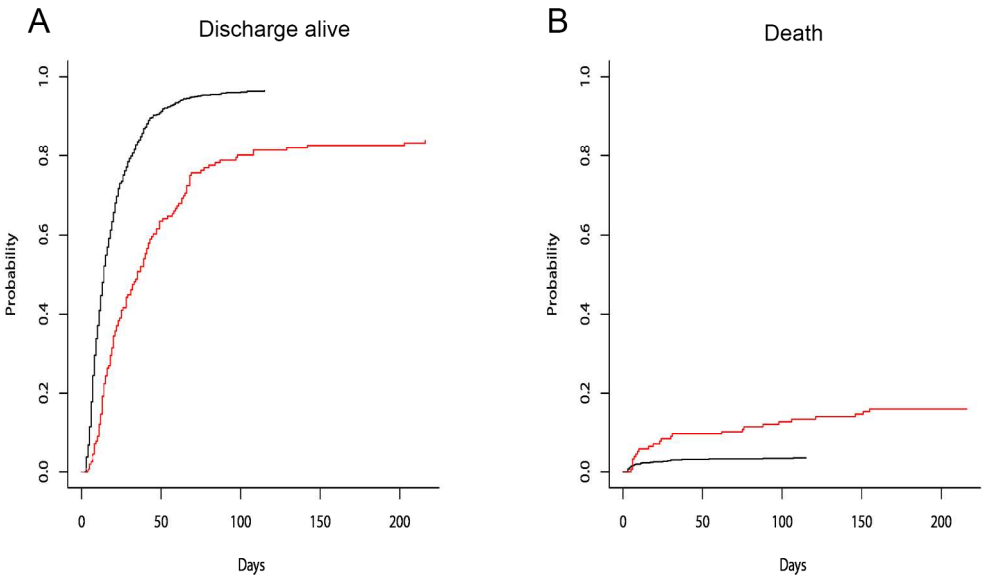


Characteristics of nosocomial infections

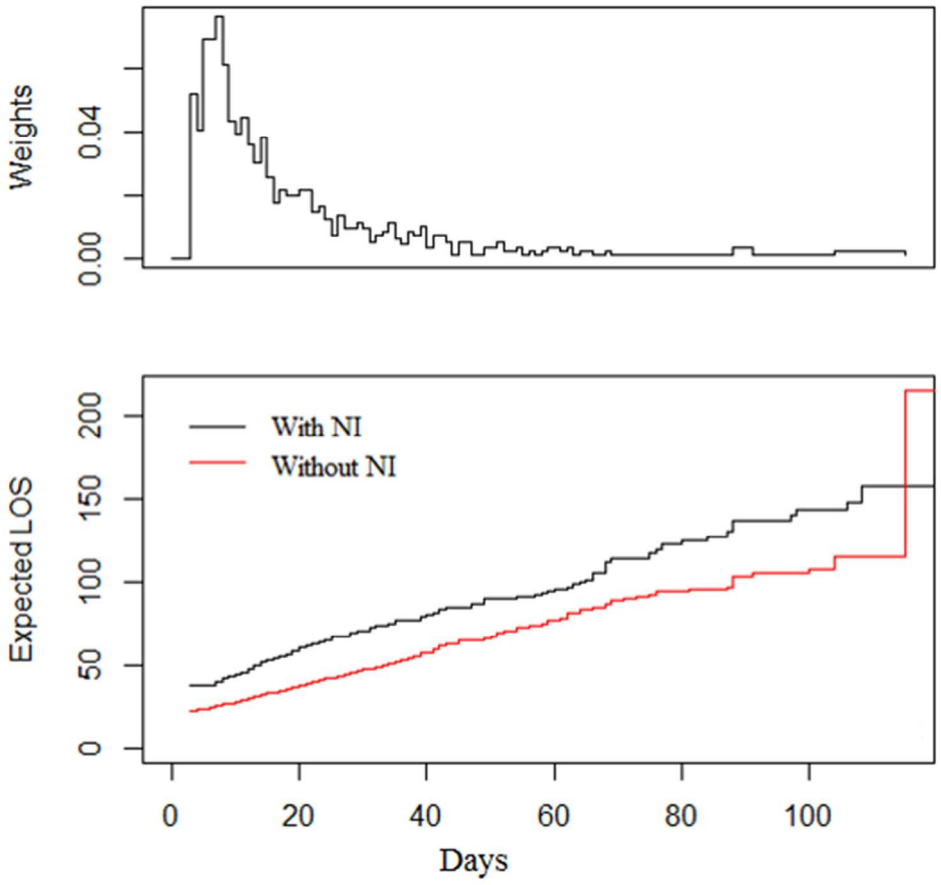
195x322mm (300 x 300 DPI)



Cumulative incidence functions for discharge (A) and death (B) in burn patients

190x127mm (300 x 300 DPI)

view only



Extra length of stay in patients without (red line) and with (black line) infection.

275x275mm (300 x 300 DPI)

Table S1. Baseline characteristics of burn patients according to survive or not.

Variables	Survivors (n=932)	Non-Survivors (n=54)	*P* value
Male, n(%)	604 (64.8%)	38 (70.4%)	0.405
Age (years), median (25th, 75th)	37 (18, 49)	40 (24, 50)	0.305
Diabetes, n (%)	36 (3.9%)	2 (3.7%)	0.953
TBSA, n (%)			
<10%	464 (49.8%)	5 (9.3%)	**0.000**
10-29%	287 (30.8%)	17 (31.5%)	0.915
⩾30%	181 (19.4%)	32 (59.3%)	**0.000**
Full thickness burn, n (%)	208 (22.3%)	13 (24.1%)	0.763
Inhalation injury, n (%)	24 (2.6%)	22 (40.7%)	**0.000**
Burn types, n (%)			
Flame	727 (78.0%)	44 (81.5%)	0.547
Scalding	89 (9.5%)	5 (9.3%)	0.944
Electric	71 (7.6%)	2 (2.7%)	0.285
Others	43 (4.6%)	3 (5.6%)	0.750
NI, n (%)	131 (14.1%)	25 (46.3%)	**0.000**

NI: nosocomial infection; TBSA: total body surface area

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STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	P2 1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	P4 2	Explain the scientific background and rationale for the investigation being reported
Objectives	P4 3	State specific objectives, including any prespecified hypotheses
Methods		
Study design	P4 4	Present key elements of study design early in the paper
Setting	P4 5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls P5 *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	P5 7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	P5-6 8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	P5-6 11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	P6-7 12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses

Continued on next page

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Results		
Participants	13*	P7 (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	P7 (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		P7 *Cross-sectional study*—Report numbers of outcome events or summary measures
Main results	P816	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	P11 18	Summarise key results with reference to study objectives
Limitations	P3,11 19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	P3 20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	P11 22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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