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ELEVATED PD-1/CD28 RATIO RATHER THAN PD-1 EXPRESSION IN CD8$^+$ T CELLS PREDICTS NOSOCOMIAL INFECTION IN SEPSIS PATIENTS: A PROSPECTIVE, OBSERVATIONAL COHORT STUDY

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ABSTRACT—Background: The expression of programmed cell death 1 receptor (PD-1) and CD28 on CD8$^+$ T cells is considered to be related to immune function and prognosis markers in patients with sepsis. However, the relationship between the ratio of PD-1/CD28 and nosocomial infection has not been elucidated. **Methods:** A prospective, observational cohort study was conducted in a general intensive care unit. Patients were enrolled according to the sepsis-3 criteria and peripheral blood samples were collected within 24 hours of enrollment. Programmed cell death 1 receptor and CD28 expression on CD8$^+$ T cells was assayed on day 1. Patients were followed up until 28 days. Multivariate regression analysis was used to assess independent risk factors for nosocomial infection. The accuracy of biomarkers for nosocomial infection and mortality was determined by the area under the receiver operating characteristic curve analysis. The association between biomarkers and 28-day mortality was assessed by Cox regression survival analysis. **Results:** A total of 181 patients were recruited, and 68 patients were finally included for analysis. Of these, 19 patients (27.9%) died during 28 days and 22 patients (32.4%) acquired nosocomial infection. The PD-1/CD28 ratio of patients with nosocomial infection was significantly higher than those without (0.27 [0.10–0.55] vs. 0.15 [0.08–0.28], $P = 0.025$). The PD-1/CD28 ratio in CD8$^+$ T cells (odds ratio, 53.33; 95% confidence interval, 2.39–1188.22, $P = 0.012$) and duration of mechanical ventilation (odds ratio, 1.14; 95% confidence interval, 1.06–1.24; $P = 0.001$) were independently associated with nosocomial infection. The area under the receiver operating characteristic curve of PD-1/CD28 ratio in CD8$^+$ T cells was 0.67 (0.52–0.82). The PD-1/CD28 ratio in CD8$^+$ T cells of the nonsurvivors was significantly higher than the survivors (0.23 [0.15–0.52] vs. 0.14 [0.07–0.32]); Cox regression analysis showed that the survival time of patients with PD-1/CD28 ratio in CD8$^+$ T cells of 0.13 or greater was shorter compared with patients with lower levels (hazard ratio, 4.42 [1.29–15.20], $\chi^2 = 6.675$; $P = 0.010$). **Conclusions:** PD-1/CD28 ratio in CD8$^+$ T cells at admission may serve as a novel prognostic biomarker for predicting nosocomial infection and mortality.

KEYWORDS—Sepsis, T lymphocyte, programmed cell death 1 receptor, CD28, nosocomial infection, prognosis

ABBREVIATIONS—APACHE II—Acute Physiology and Chronic Health Evaluation II; APC = antigen-presenting cell; AUC = area under the receiver operating characteristic curve; CI = confidence interval; GCS = Glasgow Coma Scale; HR = hazard ratio; IL-2 = interleukin-2; IQR = interquartile range; Lac = lactate; MAP = mean arterial pressure; OR = odds ratio; PD-1 = programmed cell death 1 receptor; ROC = receiver operating characteristic; SOFA = sequential organ failure assessment; WBC = white blood cell

BACKGROUND

Sepsis, defined by a dysregulated immune response to infection that leads to life-threatening organ dysfunction (1), remains a leading cause of mortality affecting approximately 48.9 million people worldwide each year (2). Nosocomial infections after sepsis are common, and they posed a higher risk of mortality compared with community-acquired infection (3). Sepsis-related immunosuppression is the main factor contributing to nosocomial

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The authors report no conflicts of interest.

This study was reviewed and approved by the Guangdong Provincial People's Hospital Ethics Committee. It was conducted in accordance with the Declaration of Helsinki (no. GDREC2015374H). Informed consent was obtained from all patients or their legal proxy before enrollment.

The data sets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

W.J. and H.Z. designed the study. W.J., W.Z., J.L., D.L., X.L., M.L., T.Z., J.H., and G.H. performed the research and collected the data. J.L., X.L., and M.Z. carried out the flow cytometric analysis. W.J., W.Z., and J.L. analyzed the data. W.J., W.Z., J.L., and M.Z. wrote the manuscript. All authors read and approved the final version of the manuscript.

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infections (4). Therefore, the prediction of nosocomial infections is deemed to be helpful to prevent its occurrence and improve the prognosis of patients with sepsis.

Recently, it has been reported that upregulated inhibitory immune cell subtype may be involved in nosocomial infections and immunosuppression (5). Programmed death 1 (PD-1), an immunoreceptor tyrosine-based inhibitory motif–containing receptor or an inhibitory member of the CD28 family, expressed on activated T cells, B cells, and monocytes and likely regulates these cell types (6), may be related to nosocomial infection in patients with sepsis. Among septic shock patients, elevated PD-1 combined with PD–ligand 1 of $CD4^+$ T cell resulted in increased nosocomial infections and mortality (7). This may be attributed to PD-1 interacted with PD-1 combined with PD–ligand 1 of antigen-presenting cells (APCs) that inhibited $CD4^+$ or $CD8^+$ T cells and reduced interleukin 2 (IL-2) production (8). The model of recurrent sepsis is often characterized by the exhaustion of T-cell phenotype with the downregulation of costimulatory molecule CD28 and upregulation of cosuppressor molecule PD-1 (5). Therefore, we hypothesized that PD-1 and CD28 may represent the negative and positive roles in regulating T-cell function, respectively, and that the PD-1/CD28 ratio may be a more accurate indicator of immune function. However, studies on the relationship between PD-1/CD28 ratio of $CD8^+$ T cells and nosocomial infection in sepsis patients have remained elusive.

In light of the above, this study sought to identify a reliable risk factor for prediction of occurrence of nosocomial infections and, furthermore, to verify the PD-1/CD28 ratio as a prognostic biomarker for nosocomial infection and death in patients with sepsis. We consider this to be essential for the management of antibiotics and reducing mortality in these patients.

METHODS

Design, setting, and patients

The present prospective observational study was conducted with approval from Guangdong Provincial People's Hospital Ethics Committee (no. GDREC2015374H) in a general intensive care unit (ICU) of Guangdong Provincial People's Hospital. Written informed consent was provided by the patient or legal proxy before enrollment. The study was performed during the period from July 2016 to January 2018.

Enrolled patients were adults meeting the sepsis-3 criteria according to the Third International Consensus Definitions for Sepsis and Septic Shock (1). Exclusion criteria were end-stage of chronic disease, and estimated survival time was less than 28 days, with autoimmune disease, immunodeficiency, malignant tumors or long-term use of immune suppressants, the onset time was more than 5 days, and consent could not be obtained.

Data and sample collection

Baseline characteristics included demographic characteristics, laboratory results, vital signs, the primary source of infections, and disease severity. They were recorded within 24 hours after admission of patients satisfying the criteria of sepsis-3 including those with confirmed or suspected infection who had the sequential organ failure assessment (SOFA) scores of 2 points or more. Meanwhile, patients were recruited according to the inclusion and exclusion criteria within 5 days of sepsis onset. The severity of illness was assessed using the Glasgow Coma Scale (GCS), Acute Physiology and Chronic Health Evaluation II (APACHE II), and SOFA scores. The patients were followed for at least 28 days after enrollment. Based on the mortality within 28 days and occurrence of nosocomial infection, participants were divided into 2 groups: the nonsurvivors group and survivors group, as well as the nonnosocomial group and nosocomial infection group. Ethylenediaminetetraacetic acid (EDTA)–anticoagulated blood was collected within 24 hours after enrollment through an indwelling central venous or by peripheral venipuncture. The expression of PD-1 and CD28 in T cells was assayed in the whole peripheral blood samples via flow cytometry within 1 hour of blood collection.

Flow cytometry

Antibodies for flow cytometric determinations were purchased from Bio-Legend (San Diego, Calif), or BD Pharmingen (Franklin Lake, NJ). Lymphocytes were identified by forward scatter and side scatter properties. The antibodies used above consisted of Per-CP/cyanine 5.5-labeled anti-CD3, PE-labeled anti-CD8, FITC-labeled anti-CD4, APC-labeled anti-PD-1 and PE/Cy7-labeled anti-CD28, and Aliquots of 100 μL of whole blood were incubated with monoclonal antibodies consisted of Per-CP/cyanine 5.5-labeled anti-CD3 (5 μL, clone HIT3a), PE-labeled anti-CD8, anti-CD4 (5 μL, clone RPA-T4), PE/Cy7-labeled anti-CD28 (5 μL, clone CD28.2), and APC-labeled anti-PD-1 (20 μL, clone MIH4) and isotype controls according to the manufacturer's recommendations. The erythrocytes were lysed, and cells evaluated by a researcher blinded to our clinical data. The samples were processed on CytoFLEX (Beckman Coulter, Inc, Brea, Calif) and analyzed using CytExpert software version 2.0 (Beckman Coulter, Inc).

Definitions

Nosocomial infection is defined as follows: (1) an infection acquired after 48 hours of patient admission to a given hospital or other healthcare institutions resulting from delivering healthcare services to patients (9); there must be no evidence that the infection was present or incubating at the time of admission; (2) a direct connection with the previous hospitalization; (3) a new infection showed up in other parts that based on the original infections (except for septic foci transfer elsewhere) or other pathogens were isolated from the primary source of infection (eliminate contamination and original mixed bacteria); and (4) potential infections activated by diagnostic and therapeutic measures, such as herpes virus and tuberculosis infection. The most common hospital-acquired infections are pulmonary infection, urinary tract infection, bloodstream infection, and catheter-related infections.

Statistical analysis

Differences in patients' clinical and biological parameters were examined using frequencies/percentage, means/standard deviations, and medians/interquartile ranges (IQRs). The comparisons between groups for continuous variables were calculated using the Mann-Whitney U test or, when categorical data were performed, the Pearson χ^2 test. Multivariate regression analysis was used to assess the independent risk factors for nosocomial infection. The patient survival was analyzed using Cox regression survival analysis. The predictive accuracy of biomarkers for nosocomial infection and 28-day mortality was determined by the area under the receiver operating characteristic curve (AUC) analysis. SPSS (version 20.0) was used for the collected data analyses. An α level of 0.05 was used for all analyses.

RESULTS

Demographic characteristics and clinical data

In total, 181 adult patients with sepsis were admitted to the ICU of Guangdong Provincial People's Hospital. Considering the prespecified criteria, 77 participants were enrolled and one patient was lost to follow-up; 9 patients were removed from the final analysis. This yielded a study cohort of 68 participants: 46 in the nonnosocomial infection group and 22 in the nosocomial infection group (Fig. 1). The median age of the patients was 70 years, ranging from 18 to 80 years. Of these, 19 died within 28 days. All patients performed bacterial cultures of blood, sputum, urine, specimens, or other infected sites: 51.5% (35/68) were positive cultures. Among patients with nosocomial infection, lung infections accounted for 59.1%, urinary system accounted for 22.7%, abdominal infections accounted for 13.6%, and bloodstream infections accounted for 4.5%. Compared with patients of nonnosocomial infection group, patients with nosocomial infection took longer mechanical ventilation. The positive rate of bacterial culture for primary infection (86.4% vs. 34.8%, $P < 0.001$) and the incidence of pneumonia (59.1% vs. 30.4%, $P = 0.024$) in patients with nosocomial infection was significantly higher than those without. The number of white blood cells, the concentrations of serum procalcitonin, lactic acid, C-reactive protein, and several types of scoring items that indicated the severity of the disease including

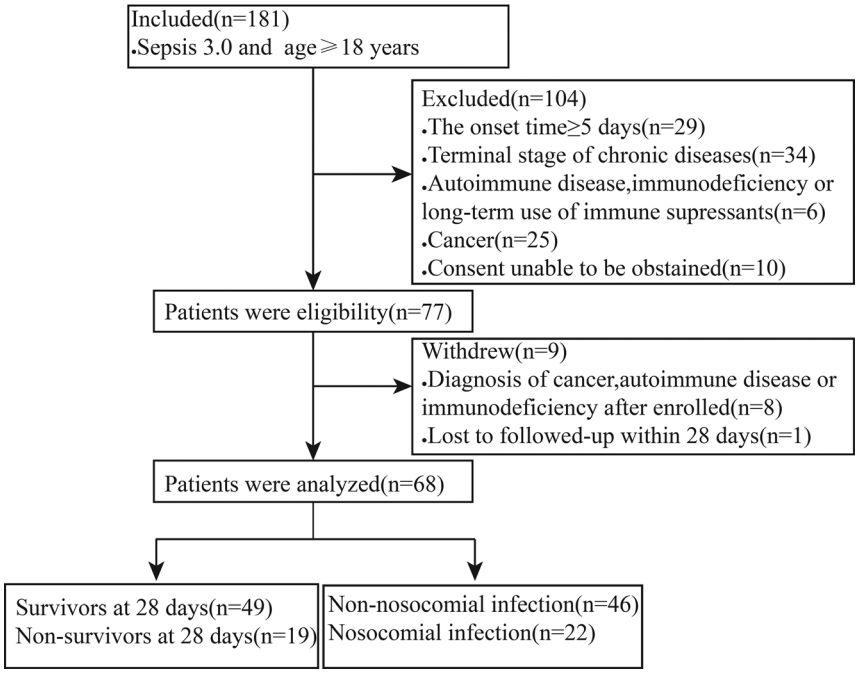


FIG. 1. **Schematic of the study.**

GCS score, APACHE II, and SOFA score showed no differences between the 2 groups. Meanwhile, the percentage of indwelling central venous catheter in the nosocomial infection group and the nonnosocomial infection group was 90.9% and 67.4%, respectively. The proportion of indwelling endotracheal intubation was 90.9% and 60.9% in the 2 groups. All *P* values were less than 0.05, indicating that indwelling central venous tube and endotracheal intubation might affect the occurrence of nosocomial infection. The demographic and clinical characteristics of the cohort were presented in Table 1.

TABLE 1. **Demographic and clinical data for sepsis patients**

Parameters	All patients (N = 68)	Nonnosocomial infection group (n = 46)	Nosocomial infection group (n = 22)	*P*
Demographic characteristics				
Female, n (%)	26 (38)	19 (41)	7 (32)	0.451
Age, y	70 (60–77)	69 (62–76)	73 (57–80)	0.974
Laboratory findings				
WBC, $\times 10^9$/L	15.32 (9.26–20.59)	16.33 (8.90–22.40)	12.28 (9.42–17.56)	0.277
Lac, mmol/L	1.4 (1.0–2.2)	1.40 (1.1–2.5)	1.7 (1.0–2.1)	0.803
CRP, mg/L	155.6 (78.8–200.1)	175.6 (88.4–90.9)	126.4 (73.7–174.0)	0.137
PCT, ng/mL	24.2 (6.5–81.4)	31.7 (6.8–90.9)	12.1 (6.1–45.1)	0.090
Bacteriology positive, n (%)	35 (51.5)	16 (34.8)	19 (86.4)	0.000
Vital signs				
T, °C	36.8 (36.5–37.2)	36.8 (36.6–37.1)	36.8 (36.5–37.4)	0.669
Heart rate, bpm	93 (83–105)	94 (84–105)	92 (82–105)	0.670
MAP, mm Hg	87.7 (80.1–99.3)	86.2 (79.7–100.2)	87.8 (81.0–98.6)	0.694
The primary source of infection, n (%)				
Bloodstream	4 (5.9)	3 (6.5)	1 (4.5)	1.000
Lungs	27 (39.7)	14 (30.4)	13 (59.1)	0.024
Abdomen	20 (29.4)	17 (37.0)	3 (13.6)	0.048
Urinary system	16 (23.5)	11 (23.9)	5 (22.7)	0.914
Other	1 (1.5)	1 (2.2)	0 (0.0)	1.000
Severity of illness				
GCS score	11 (6–15)	14 (7–15)	10 (3–15)	0.103
APACHE II score	16 (11–24)	15 (10–23)	21 (14–24)	0.128
SOFA score	7 (5–10)	7 (4–9)	9 (6–12)	0.064
Duration of mechanical ventilation, d	5 (0–13)	2 (0–6)	14 (7–26)	0.000
Indwelling catheters, n (%)	63 (92.6)	41 (89.1)	22 (100.0)	0.267
Indwelling the central venous catheter, n (%)	51 (75.0)	31 (67.4)	20 (90.9)	0.036
Indwelling the endotracheal intubation, n (%)	48 (70.6)	28 (60.9)	20 (90.9)	0.011

Data are shown as the frequencies/percentage and medians/IQRs, unless otherwise indicated. χ^2 test or Fisher exact test was performed for categorical data and nonparametric Mann-Whitney *U* test for continuous variables.

CRP, C-reactive protein; Lac, lactate; MAP, mean arterial pressure; PCT, procalcitonin; T, temperature; WBC, white blood cell.

TABLE 2. **Comparison of lymphocyte and cosignaling molecules on T cells between the nosocomial and nonnosocomial infection group**

Parameters	All patients (N = 68)	Nonnosocomial infection group (n = 46)	Nosocomial infection group (n = 22)	*P*
WBC, $\times 10^9$/L	15.32 (9.26–20.59)	16.33 (8.90–22.40)	12.28 (9.42–17.56)	0.277
N, $\times 10^9$/L	12.55 (7.85–19.10)	14.16 (7.73–20.54)	11.06 (7.80–17.11)	0.277
Lymphocyte, $\times 10^9$/L	0.78 (0.38–1.19)	0.84 (0.40–1.18)	0.62 (0.32–1.24)	0.516
T lymphocyte, $\times 10^9$/L	0.36 (0.12–0.75)	0.33 (0.13–0.74)	0.37 (0.06–0.86)	0.783
No. $CD4^+$ T cells, $\times 10^9$/L	0.13 (0.05–0.34)	0.13 (0.05–0.30)	0.12 (0.04–0.38)	0.865
No. $CD8^+$ T cells, $\times 10^9$/L	0.08 (0.02–0.20)	0.10 (0.04–0.21)	0.07 (0.02–0.18)	0.332
CD4/CD8 ratio	1.6 (1.0–2.7)	1.6 (1.0–2.3)	1.7 (1.2–3.4)	0.110
CD28 in $CD4^+$ T cells, %	94.4 (88.9–97.3)	93.9 (89.1–97.4)	94.6 (87.6–97.0)	0.829
CD28 in $CD8^+$ T cells, %	52.9 (29.3–72.5)	51.2 (38.6–73.3)	56.8 (17.7–69.4)	0.521
PD-1 in $CD4^+$ T cells, %	7.4 (5.2–11.3)	7.6 (5.5–11.1)	6.5 (4.4–11.8)	0.326
PD-1 in $CD8^+$ T cells, %	8.4 (4.5–12.8)	7.5 (4.00–12.5)	9.4 (7.2–14.9)	0.183
PD-1/CD28 ratio in $CD4^+$ T cells	0.08 (0.06–0.12)	0.08 (0.06–0.12)	0.07 (0.04–0.14)	0.587
PD-1/CD28 ratio in $CD8^+$ T cells	0.17 (0.08–0.33)	0.15 (0.08–0.28)	0.27 (0.10–0.55)	0.025

Data are shown as median and IQR, unless otherwise indicated. All the above were performed on a nonparametric test of 2 independent samples (Mann-Whitney). N, the accounts of neutrophil; WBC, the accounts of white blood cell.

Comparison of blood cells, lymphocyte subsets, and expression of cosignaling molecules on T cells between the nosocomial infection and the nonnosocomial infection group

The PD-1/CD28 ratio in $CD8^+$ T cells was significantly higher in the nosocomial infection group than the nonnosocomial infection group (0.27 [0.10–0.55] vs. 0.15 [0.08–0.28], $P = 0.025$). No significant differences were detected between the 2 groups with respect to the counts of white blood cells, neutrophils, lymphocytes, T lymphocytes, $CD4^+$ T cells and $CD8^+$ T cells, CD4/CD8 ratio, CD28 in $CD8^+$ T cells, PD-1 in $CD8^+$ T cells, CD28 in $CD4^+$ T cells, PD-1 in $CD4^+$ T cells, and PD-1/CD28 ratio in $CD4^+$ T cells (Table 2, Fig. 2A). A similar pattern was seen in nonsurvival group and survival group. In addition, the percentage of $PD\text{-}1^+$ in $CD8^+$ T cells (11.4 [7.7–15.9] vs. 7.5 [3.8–11.6], $P = 0.015$) was significantly higher in the nonsurvivors than the survivors (Table 3).

Correlation between the PD-1/CD28 ratio in $CD8^+$ T cells and nosocomial infection and 28-day mortality

The ratio of PD-1/CD28 in $CD8^+$ T cells of sepsis patients was significantly increased in the nosocomial as compared with the nonnosocomial groups (Fig. 2B). Meanwhile, sepsis patients with poor prognosis showed a significant increase in ratio of PD-1/CD28 in $CD8^+$ T cells in comparison with that in patients with favorable outcome (Fig. 2C).

The PD-1/CD28 ratio in $CD8^+$ T cells as an independent predictor of nosocomial infection in sepsis patients

In a univariable logistic regression analysis, PD-1/CD28 ratio in $CD8^+$ T cells and duration of mechanical ventilation were significantly related to nosocomial infection. Furthermore, multivariable logistic analysis revealed that the PD-1/CD28 ratio in $CD8^+$

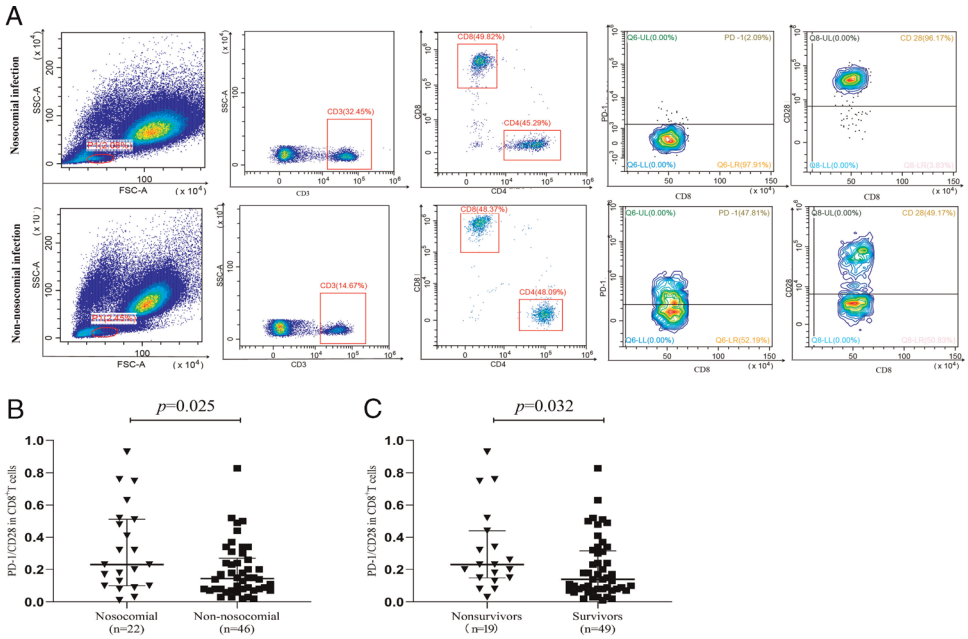


FIG. 2. **The ratio of PD-1/CD28 in $CD8^+$ T cells in the derivation cohort of patients with sepsis.** A, Representative flow histograms of lymphocyte gating strategy. The PD-1 and CD28 in $CD8^+$ T cells between nosocomial and nonnosocomial of sepsis patients. B, Dot plots show the ratio of PD-1/CD28 in $CD8^+$ T cells of sepsis patients to ascertain the statistical differences between the nosocomial and nonnosocomial groups. The samples had a significantly higher ratio of PD-1/CD28 (0.27 [0.10–0.55] vs. 0.15 [0.08–0.28], $P = 0.025$) in $CD8^+$ T cells in nosocomial as compared with nonnosocomial. C, Dot plots show the ratio of PD-1/CD28 in $CD8^+$ T cells of sepsis patients to ascertain the statistical differences between the nonsurvivors and survivors group. The samples had a significantly higher ratio of PD-1/CD28 (0.23 [0.15–0.52] vs. 0.14 [0.07–0.32], $P = 0.032$) in $CD8^+$ T cells in the nonsurvivors compared with the survivors.

TABLE 3. **Comparison of lymphocyte and cosignaling molecules on T cells between the survivor and nonsurvivor groups**

Parameters	All patients (N = 68)	Survivors (n = 49)	Nonsurvivors (n = 19)	*P*
WBC, $\times 10^9$/L	15.32 (9.26–20.59)	15.99 (9.55–20.77)	11.95 (8.13–17.36)	0.178
N, $\times 10^9$/L	12.55 (7.85–19.10)	13.80 (8.20–19.51)	11.16 (6.64–16.93)	0.194
Lymphocyte, $\times 10^9$/L	0.78 (0.38–1.19)	0.81 (0.47–1.16)	0.40 (0.25–1.37)	0.271
T lymphocyte, $\times 10^9$/L	0.36 (0.12–0.75)	0.38 (0.13–0.76)	0.27 (0.08–0.52)	0.296
No. $CD4^+$ T cells, $\times 10^9$/L	0.13 (0.05–0.34)	0.13 (0.05–0.33)	0.10 (0.02–0.36)	0.657
No. $CD8^+$ T cells, $\times 10^9$/L	0.08 (0.02–0.20)	0.08 (0.03–0.19)	0.07 (0.02–0.30)	0.608
CD4/CD8 ratio	1.60 (0.97–2.66)	1.47 (0.94–2.71)	1.72 (1.22–2.34)	0.482
CD28 in $CD4^+$ T cells, %	94.4 (88.9–97.3)	92.6 (88.5–97.0)	95.0 (91.2–97.4)	0.229
CD28 in $CD8^+$ T cells, %	52.9 (29.3–72.5)	50.4 (30.3–66.6)	63.5 (27.5–78.1)	0.401
PD-1 in $CD4^+$ T cells, %	7.4 (5.2–11.3)	7.0 (4.7–10.3)	7.5 (6.0–11.8)	0.302
PD-1 in $CD8^+$ T cells, %	8.4 (4.5–12.8)	7.5 (3.8–11.6)	11.4 (7.7–15.9)	0.015
PD-1/CD28 ratio in $CD4^+$ T cells	0.08 (0.06–0.12)	0.07 (0.05–0.12)	0.09 (0.06–0.15)	0.287
PD-1/CD28 ratio in $CD8^+$ T cells	0.17 (0.08–0.34)	0.14 (0.07–0.32)	0.23 (0.15–0.52)	0.032

Data are shown as median and IQR, unless otherwise indicated. All the above were performed on a nonparametric test of 2 independent samples (Mann-Whitney). N, the accounts of neutrophil; WBC, the accounts of white blood cell.

T cells (odds ratio [OR], 53.33; 95% confidence interval [CI], 2.39–1188.22, $P = 0.012$) and duration of mechanical ventilation (OR, 1.14; 95% CI, 1.06–1.24; $P = 0.001$) were independent predictors of nosocomial infection (Table 4), allowing differentiation of patients with nosocomial infection from those without.

Predictive performance of the PD-1/CD28 ratio in $CD8^+$ T cells for nosocomial infection and 28-day mortality in sepsis patients

The area under the receiver operating characteristic (ROC) curve (AUC) of the PD-1/CD28 ratio in $CD8^+$ T cells and SOFA score of patients for predicting nosocomial infection were 0.67 (95% CI, 0.52–0.82; $P = 0.025$) and 0.65 (95% CI, 0.51–0.79; $P = 0.045$; Fig. 3A), respectively. However, the accounts of white blood cells, neutrophils, PD-1 in $CD8^+$ T cells, and the ratio of $CD4^+/CD8^+$ T cells could not predict nosocomial infection. The cutoff value of the PD-1/CD28 ratio in $CD8^+$ T cells was 0.32 with a sensitivity of 50% and a specificity of 80%. Meanwhile, the cutoff value of the SOFA score was 8.50 with a sensitivity of 54.5% and a specificity of 71.7%. In addition, the AUC of the PD-1/CD28 ratio in $CD8^+$ T cells and SOFA score for predicting 28-day mortality were 0.67 (95% CI, 0.53–0.81; $P = 0.03$) and 0.67 (95% CI, 0.52–0.83; $P = 0.028$; Fig. 3B). The cutoff value of the PD-1/CD28 ratio in $CD8^+$ T cells was 0.13 with a sensitivity of 84.2% and a specificity of 50%, and the SOFA score was 8.50 with 63.2% and 73.5%. According to the best cutoff point for predicting 28-day mortality by the PD-1/CD28 ratio in $CD8^+$ T cells, patients were divided into PD-1/CD28 ratio in $CD8^+$ T cells greater than or equal to 0.13 group and the ratio less than 0.13 group. The APACHE II score on day 7 was significantly higher in the PD-1/CD28 ratio group greater than or equal to 0.13 than the ratio less than 0.13 group (15 [12–24] vs. 10 [6–16], $P = 0.028$). A similar trend was seen about SOFA score on day 7 in above groups. However, there was no correlation between the 2 groups and ICU length of stay, nosocomial infection, and duration of mechanical ventilation of patients with sepsis.

Survival

Cox regression analysis survival curves showed the sepsis patients with the ratio of PD-1/CD28 in $CD8^+$ T cells higher than 0.13 had higher mortality at 28 days (HR, 4.42 [1.29–15.20], $\chi^2 = 6.675$, $P = 0.012$) as compared with the patients with lower levels (Fig. 4).

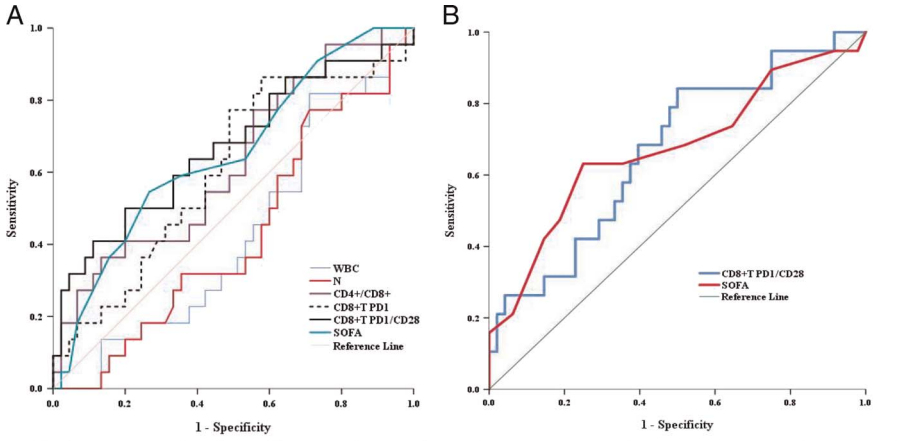
DISCUSSION

The fluctuation of T-cell coinhibitory and costimulatory molecules as one falling and the other rising may reflect the immune balance of sepsis patients. This study is the first to explore the use of the PD-1/CD28 ratio in $CD8^+$ T cells for differentiating patients with nosocomial infection among patients with sepsis in a comprehensive ICU. We show here that elevated PD-1/CD28 ratio rather than PD-1 and CD28 expression separately in $CD8^+$ T cells predicts nosocomial infection and mortality in sepsis patients. This was based on the following findings and arguments: (1) the PD-1/CD28 ratio in $CD8^+$ T cells was significantly higher in the nosocomial infection group than the nonnosocomial infection group. (2) Binary logistic regression analysis showed that PD-1/CD28 ratio in $CD8^+$ T cells and duration of mechanical ventilation were independently associated with nosocomial infection. (3) The percentage of $PD\text{-}1^+$ in $CD8^+$ T cells was significantly

TABLE 4. **Univariable and multivariable logistic analyses for predicting nosocomial infection in sepsis patients**

Variable	Univariable OR	95% CI	*P*	Multivariable OR	95% CI	*P*
Sex	1.51	0.52–4.41	0.453			
Age	1.00	0.96–1.04	0.985			
WBC	0.96	0.89–1.02	0.165			
N	0.95	0.89–1.02	0.175			
CD4/CD8 ratio	1.34	0.93–1.94	0.112			
PD-1 in $CD8^+$ T cells, %	1.05	0.99–1.12	0.122			
PD-1/CD28 ratio in $CD8^+$ T cells	19.81	1.53–256.78	0.022	53.33	2.39–1188.22	0.012
Duration of mechanical ventilation	1.11	1.04–1.18	0.001	1.14	1.06–1.24	0.001
SOFA score	1.12	0.97–1.29	0.114			

N, the accounts of neutrophil; WBC, the accounts of white blood cell.



Variable	AUC(95%CI)	*p* value
WBC	0.43(0.29-0.59)	0.336
N	0.43(0.29-0.57)	0.336
$CD4^+/CD8^+$	0.63(0.48-0.77)	0.098
$CD8^+$T PD-1	0.61(0.46-0.75)	0.159
$CD8^+$T PD-1/CD28	0.67(0.52-0.82)	0.025
SOFA	0.65(0.51-0.79)	0.045

Variable	AUC(95%CI)	*p* value
$CD8^+$T PD-1/CD28	0.67(0.53-0.81)	0.032
SOFA	0.67(0.52-0.83)	0.028

FIG. 3. **The ROC analyses for predicting nosocomial infection and 28-day mortality.** A, The PD-1/CD28 ratio in $CD8^+$ T cells and SOFA score can predict nosocomial infection but the accounts of white blood cell and neutrophil granulocyte, the percentage of PD-1^+ in $CD8^+$ T cells, and the ratio of $CD4^+/CD8^+$ not. B, The PD-1/CD28 ratio in $CD8^+$ T cells and SOFA score can predict 28-day mortality. WBC, the accounts of white blood cell; N, the accounts of neutrophil; $CD4^+/CD8^+$, the ratio of $CD4^+/CD8^+$ T lymphocytes; $CD8^+$ T PD-1, the percentage of PD-1^+ in $CD8^+$ T cells.

higher in the nonsurvivors than the survivors; indeed, the higher the PD-1/CD28 ratio in $CD8^+$ T cells, the shorter the survival time.

Immunosuppression is one of the main mechanisms leading to poor prognosis of sepsis (10). The immunosuppressive molecules, such as PD-1, with increased expression on various types of immune cells, are one of the important mechanisms underlying immunosuppression in sepsis (11,12). It has been reported that the higher the percentage of PD-1^+ monocytes, the higher the possibility of nosocomial infection in patients with sepsis (7). However, PD-1 expression on $CD4^+$ or $CD8^+$ T cells had no effect on nosocomial infection as shown in the study, and this corroborates with finding in a previous report by Wilson et al. (13). CD28 is a costimulatory molecule expressed on the surface of T lymphocytes and plays an important role in T-cell activation. The low percentage of $CD28^+$ cells in bone marrow transplant patients suggests immune dysfunction (14). However, Monserrat et al. reported that lower numbers of circulating $CD3^+CD8^+CD28^+$T cells are associated with a better disease outcome (15). Our results showed no correlation between CD28 expression on T cells and nosocomial infection. These seemingly contradictory results may stem from the fact that PD-1 and CD28 represent 2 sides of the same coin for immune function, and it would be facile to consider them separately given the complexities of their functions. It has been reported that PD-1 suppresses T-cell function

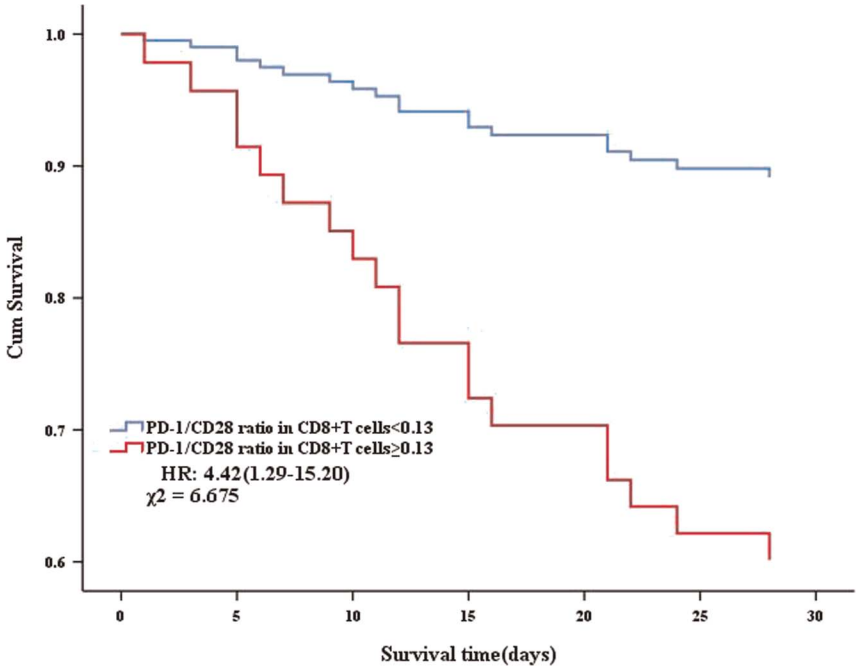


FIG. 4. **Survival curves.** Cox regression analysis survival curves showed that the survival time of patients with PD-1/CD28 ratio in $CD8^+$ T cells of 0.13 or greater was shorter compared with patients with lower levels (HR, 4.42 [1.29–15.20], $\chi^2 = 6.675$, $P = 0.010$).

primarily by inactivating CD28 signaling (16). The PD-1 blockade improves $CD8^+$ T cell differentiation and proliferation (17,18). However, CD28 has been required for $CD8^+$ T-cell proliferation after PD-1 blockade (19). This may be one of the orchestral mechanisms by which PD-1 and CD28 regulate immune function in sepsis. The finding by He et al. showed that increased PD-1 expression and reduced CD28 expression on $CD4^+$ T cells decrease antiviral immune responses (5). The percentage of PD-1^+CD28^- cells may better represent the immune status of patients (5). Thus, the PD-1/CD28 ratio can reflect the change of percentage of PD-1^+CD28^- cells to a certain extent. Of note, it was apparently easier to obtain the PD-1/CD28 ratio than PD-1^+CD28^- cell count.

Previous studies have shown that sepsis leads to lasting changes in phenotype and function of $CD8^+$ T cells (20). Cytotoxic $CD8^+$ T cells are major killers of pathogens and neoplastic cells (21). $CD8^+$ T-cell impairment due to sepsis can predispose individuals to reinfection. The PD-1/CD28 ratio in $CD8^+$ T cells was significantly elevated as a predictor for the second infection and death of sepsis patients in our study. It was reported that PD-1 inhibited IL-2 production by T cells (8); $CD8^+$ T cells, compared with $CD4^+$ T cells, were more sensitive to inhibition by the PD-1 because of their inability to produce significant levels of IL-2 (8). However, $CD8^+$ T-cell exhaustion may be reversed by IL-2 (22). This may be the reason why the PD-1/CD28 ratio is more sensitive in $CD8^+$ T cells as a predictor of nosocomial infection than in $CD4^+$ T cells.

The present study indicated that PD-1/CD28 ratio in $CD8^+$ T cells and duration of mechanical ventilation were independently associated with nosocomial infection, and it showed that the highest incidence of nosocomial infection is pulmonary infections, followed by urinary tract infections, and bloodstream infections, and the incidence of pulmonary infections was higher than that in other studies (23,24). This, to some extent, explains why the duration of mechanical ventilation was an independent risk factor for nosocomial infections. In the prediction of nosocomial infection, the efficiency of PD-1/CD28 ratio in $CD8^+$ T cells is more effective than SOFA scoring; with regard to 28-day mortality, the reverse is the case. The evaluation items of the SOFA score exclude the immune function, so the inclusion of indicators reflecting the immune function into the SOFA scoring system might further optimize the predictive efficacy of SOFA scores (25). Thus, PD-1/CD28 ratio in $CD8^+$ T cells may be used as a candidate indicator or combined with SOFA scores to make up the deficiency of SOFA scores to predict the 28-day mortality.

The current results provide an important predictive factor for nosocomial infection and mortality after sepsis, but the study has some limitations. First, this is a single-center study and our sample size was relatively small. Second, although this study showed that lymphocytes from patients with sepsis had phenotypic markers consistent with immunosuppression and T-cell exhaustion, but we did not evaluate the changes of the markers over time after sepsis; moreover, cell function studies were not conducted. Therefore, we do not know the extent or intensity of T-cell impairment in individual patients. Third, the phenotypic distribution of PD-1 and CD28 on individual T cells was not assessed in details. Although we have shown that changes in the PD-1/CD28 ratio were related to nosocomial infection, it cannot fully reflect the phenotypic information of each cell.

CONCLUSIONS

The study showed that the PD-1/CD28 ratio rather than PD-1 expression in $CD8^+$ T cells may represent an early diagnostic predictor for nosocomial infection and mortality in patients with sepsis.

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