

# Expression of PD-1 on Memory T Lymphocytes Predicts 28-Day Mortality of Patients with Sepsis: A Prospective Observational Study

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**Background:** PD-1 is an important immune checkpoint expressed on T lymphocytes and is associated with T-cell function in sepsis. However, the role of PD-1 in naive and memory T-cell responses in sepsis is not well understood. We aimed to determine the expression of PD-1 induced on naive and memory T lymphocytes in patients with sepsis and its association with clinical outcome.

**Methods:** A prospective observational study was conducted at a general intensive care unit (ICU). Whole blood samples were collected from patients within 48 h after sepsis diagnosis. PD-1 expression on naive and memory T cells was measured by flow cytometry. The levels of IFN- $\gamma$ , IL-2 and TNF- $\alpha$  released by memory T cells were also determined. All patients were followed up to 28 days, and 28-day mortality was recorded.

**Results:** PD-1 expression showed no difference in naive CD4<sup>+</sup> T cells ( $P=0.617$ ) or naive CD8<sup>+</sup> T cells ( $P=0.079$ ) between survivors ( $n = 21$ ) and nonsurvivors ( $n = 9$ ). Increased PD-1 expression on memory CD4<sup>+</sup> T cells was found in nonsurvivors ( $P=0.030$ ) and memory CD8<sup>+</sup> T cells ( $P=0.006$ ) in comparison with survivors. According to the cutoff value of the percentage of PD-1 on memory CD8<sup>+</sup> T cells in predicting 28-day mortality of patients with sepsis, patients were divided into two groups. The 28-day mortality rates between the two groups were significantly different ( $P=0.009$ ). A Kaplan Meier curve was constructed to derive a hazard ratio of 9.33 (95% CI: 2.52–34.60) for the percentage of PD-1 on memory CD8<sup>+</sup> T cells regarding 28-day mortality. In addition, the IFN- $\gamma$  secretion of memory CD4<sup>+</sup> T cells ( $P=0.046$ ) and IL-2 secretion of memory CD8<sup>+</sup> T cells ( $P=0.014$ ) were significantly greater in survivors than nonsurvivors.

**Conclusion:** Flow cytometric assessment of PD-1 expression on memory CD8<sup>+</sup> T cells identifies patients with poor outcomes during sepsis.

**Keywords:** PD-1, memory T cells, sepsis, outcome

## Background

Sepsis, caused by a dysregulated host response to infection, is a life-threatening condition with organ dysfunction.<sup>1</sup> Approximately 270,000 to 370,000 people die from sepsis every year in the US, making sepsis the leading cause of death for patients with critical illness.<sup>2</sup> In China, one-fifth of patients are admitted to the intensive care unit (ICU) with sepsis and the 90-day mortality rate is as high as 35.5%.<sup>3</sup> The treatment of sepsis remains a major challenge, and there is currently no FDA-approved therapy for sepsis.<sup>4</sup> Even if survival from the cytokine storm improves, previously septic individuals still face a higher risk of mortality, which defines the sepsis-induced immunosuppressive state.<sup>5,6</sup>

Sepsis-induced immunosuppression is characterized by an increased sensitivity to new infections unrelated to previous encounters.<sup>6</sup> These severe lesions were sufficient to reduce 5-year survival in the septic cohort compared to the nonseptic cohort.<sup>7</sup> T cells play an important role in the host immune response in sepsis. Sepsis-induced immunologic impairment is typified by a transient reduction in lymphocytes and a decrease in the potency of effector lymphocytes.<sup>8,9</sup> Specifically, lymphopenia caused by sepsis affects both naive T cells and memory T cells. Memory T cells are more

susceptible than naive T cells to sepsis-induced cell loss.<sup>10,11</sup> Additionally, memory T cells that survived the cytokine storm were less able to respond to inflammatory signals,<sup>12</sup> suggesting that memory T cells may be a crucial factor in the pathogenic mechanism of sepsis.

The balance of coinhibitory molecules and costimulatory molecule signals that T cells receive determines the fate of T-cell activation.<sup>13</sup> Both naive and memory T cells are effectively regulated by ligation of inhibitory receptors on the cell surface,<sup>14</sup> which have negative immune regulation in the T-cell response during sepsis.<sup>15–17</sup> Programmed cell death 1 (PD-1) was found to be increased on the surface of T cells in patients with sepsis, rather than T cells from nonseptic controls,<sup>18</sup> and blocking the PD-1/PD-L1 pathway may represent a potential therapeutic strategy for the amelioration of sepsis.<sup>19</sup> However, the role of PD-1 in naive T cells and memory T cells in sepsis is not clear. Thus, we enrolled patients with sepsis and examined the expression of PD-1 on naive and memory T cells, to elucidate the relationship between PD-1 expression on naive and memory T cells and the outcome of septic patients.

## Methods

### Patients

A prospective observational study was conducted by enrolling consecutive adult patients admitted to the ICU of Zhongda Hospital affiliated to Southeast University from December 2021 to March 2022. The inclusion criterion was the presence of sepsis as defined by the Sepsis-3.0 criteria.<sup>1</sup> The exclusion criteria included the following: patients younger than 18 years; pregnancy; patients with active malignancy, or chronic viral infection, such as HIV, hepatitis B or C; autoimmune diseases, use of corticosteroid therapy (except for previous use of prednisone <10 mg or equivalent per day); and length of stay in hospital lasting for more than 1 week before ICU admission or consent could not be obtained. All patients were evaluated after admission and administered therapy according to the 2016 international guidelines for the management of sepsis.<sup>4</sup> The study was approved by Zhongda Hospital affiliated to Southeast University Ethics Committee (Southeast University, Nanjing, China, approval ID: 2020ZDSYLL041-Y01) and was in full compliance with the Declaration of Helsinki. Written informed consent was obtained from each patient or their legal representative prior to enrollment in the study.

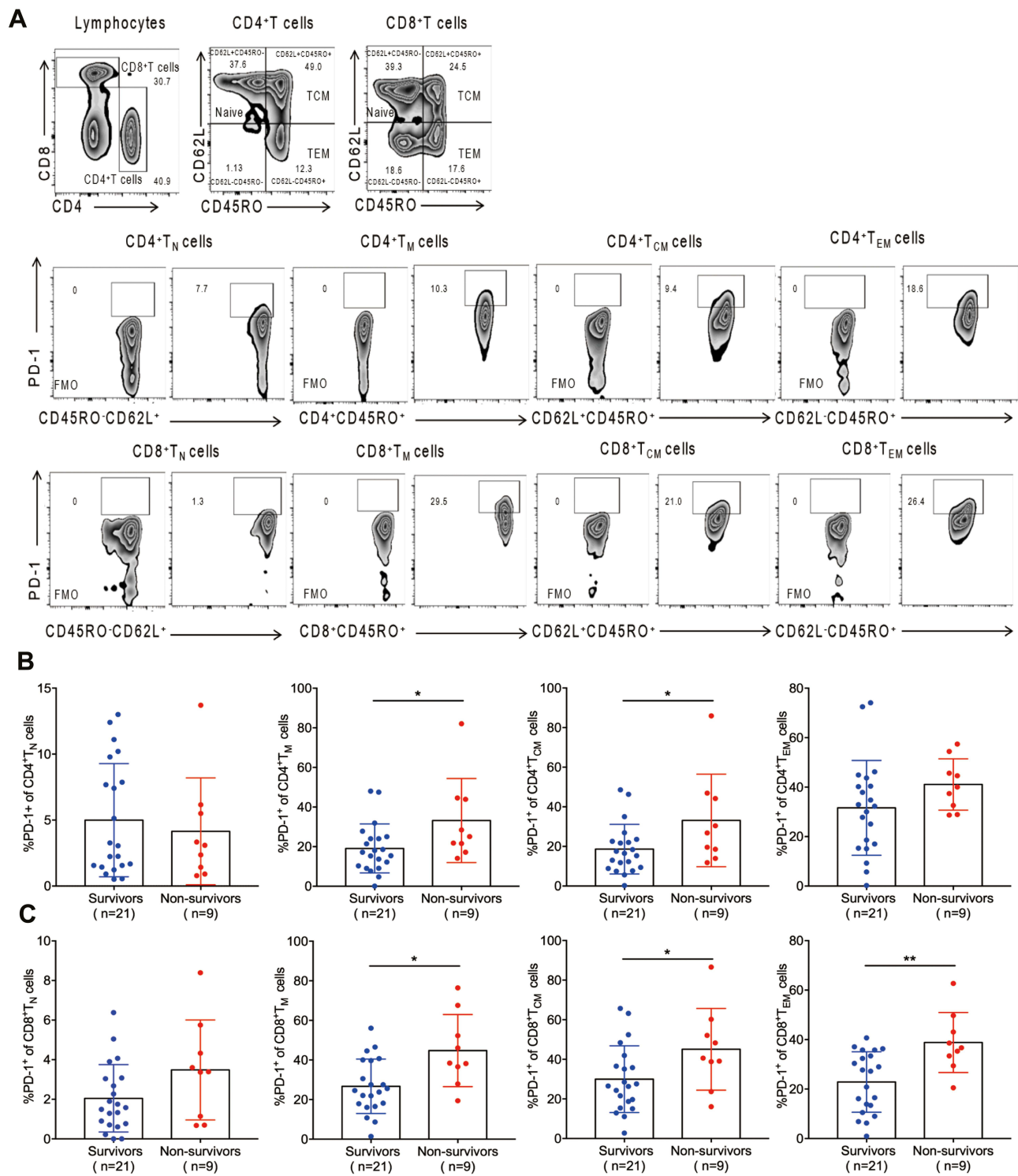
### Data Collection and Definition

Demographic characteristics including age, sex, height and weight were recorded at admission. Clinical features, including the source of infection, acute physiology and chronic health evaluation II (APACHE II) score, sequential organ failure assessment (SOFA) score and comorbidities were recorded. Inflammatory markers such as procalcitonin (PCT) and c-reactive protein (CRP) were also recorded. Records of mechanical ventilation (MV), continuous renal replacement therapy (CRRT) and vasopressor were collected. All patients were followed up to 28 days.

### Flow Cytometry

Whole blood samples were collected from patients within 48h after sepsis diagnosis. Cells were identified by forward scatter (FSC) and side scatter (SSC) properties and lymphocytes were identified with anti-CD45-Alexa Fluor<sup>®</sup>700 (clone H130, BioLegend. USA), anti-CD4-FITC (clone RPA-T4, BioLegend. USA), anti-CD8-APC-H7 (clone SK1, BD Pharmingen<sup>™</sup>. USA), anti-CD62L-Brilliant Violet 605<sup>™</sup> (clone DREG-56, BioLegend. USA), anti-CD45RO-Brilliant Violet 650<sup>™</sup> (clone UCHL1, BioLegend. USA), anti-PD-1-BV786 (clone EH12.1, BD Horizon<sup>™</sup>. USA), anti-CD28-Percp-cy5.5 (clone CD28.2, BioLegend. USA), anti-CD25-PE/Dazzle<sup>™</sup>594 (clone BC96, BioLegend. USA) and anti-TIGIT-Brilliant Violet 510<sup>™</sup> (clone A15153G, BioLegend. USA) were used for surface staining and anti-CTLA-4-Brilliant Violet 421<sup>™</sup> (clone BNI3, BioLegend. USA) was used for intracellular staining to determine T-cell phenotype. The gating strategy is shown in [Figure 1A](#). CD45RO<sup>-</sup>CD62L<sup>+</sup> T cells represent naive T cells in humans. CD62L<sup>-</sup>CD45RO<sup>+</sup> T cells and CD62L<sup>+</sup>CD45RO<sup>+</sup> T cells represent effector memory T cells and central memory T cells, respectively. PD-1 expression on memory T cells was defined using the PD-1 fluorescence minus one (FMO) strategy.

For intracellular cytokine staining, 250  $\mu$ L of blood was stimulated with cell activation cocktail (with brefeldin A) (eBioscience) for 5 h at 37°C. After this, the cells were stained with anti-CD45, anti-CD3-Percp-cy5.5 (clone OKT3, BioLegend. USA), anti-CD8 and anti-CD45RO, and then fixation and permeabilization solution (eBioscience) was used



**Figure 1** PD-1 expression on memory T cells was associated with 28-day mortality in septic patients. **(A)** Gating strategy used for flow cytometric analysis. **(B)** Percentage of PD-1 expression on naive CD4<sup>+</sup> T cells, memory CD4<sup>+</sup> T cells, central memory CD4<sup>+</sup> T cells and effector memory CD4<sup>+</sup> T cells between survivors and nonsurvivors. **(C)** Percentage of PD-1 expression on naive CD8<sup>+</sup> T cells, memory CD8<sup>+</sup> T cells, central memory CD8<sup>+</sup> T cells and effector memory CD8<sup>+</sup> T cells between survivors and nonsurvivors. \*p < 0.05, \*\*p < 0.01.

to permeabilize the cells. To stain intracellular cytokines, we used anti-IL-2-Brilliant Violet 510™ (clone MQ1-17H12, BioLegend, USA), anti-IFN-γ-Alexa Fluor® 488 (clone B27, BD Pharmingen™, USA) and anti-TNF-α-Brilliant Violet 421 (clone Mab11, BD Pharmingen™, USA). Samples were acquired using a BD FACSCelesta™ flow cytometer (BD Biosciences, USA). The data was analyzed using FlowJo software.

## Statistical Analysis

Continuous variables with a normal distribution were presented as the mean and standard deviation (SD) and were compared with Student's *t* test. Continuous variables with a skewed distribution were expressed as intermediate values within a quartile and were analyzed with the Mann–Whitney *U*-test. Categorical variables were expressed as frequencies and compared with the  $X^2$  test or Fisher's exact test.

Receiver operating characteristic (ROC) curves were used to estimate the accuracy of the level of PD-1 expressed on memory T lymphocytes for 28-day mortality predictions with the area under the ROC (AuROC) curve and the cutoffs optimized using the Youden J statistic. Discrepancies in the 28-day mortality rates were analyzed with the Log rank test and expressed with Kaplan–Meier curves.

A *P* value <0.05 was considered as statistically significant. All statistical analyses were performed using SPSS 25 software (IBM SPSS, USA), MedCalc (version 19.6.1) and GraphPad Prism 7.0 (GraphPad Software, San Diego, USA).

## Results

### Patient Characteristics

A total of 66 patients with sepsis were screened and 30 patients were enrolled. One patient was pregnant. Additionally, 1 patient with hepatitis C virus infection, 8 patients with active malignancy, 1 patient with autoimmune disease, 3 patients who received the corticosteroid treatment and 22 patients who had been in the hospital for more than one week were excluded. The flow diagram of study patients is presented in [Figure S1](#). The mean age of these patients was  $64.70 \pm 13.95$  years and 21 (70.0%) of them were male. The most common comorbidity was hypertension ( $n = 15$ , 50.0%), followed by diabetes ( $n = 2$ , 7.0%). The leading cause of sepsis was abdominal infection ( $n = 14$ , 47.0%), followed by pulmonary infection ( $n = 13$ , 43.0%). All patients were followed up to 28 days, at which time there were 21 survivors and 9 nonsurvivors. The overall 28-day mortality was 30.0%.

The clinical characteristics of survivors and nonsurvivors are described in [Table 1](#). There were no significant differences in age, sex, body mass index (BMI), comorbidities, source of infection, utilization rate of vasoactive drugs, mechanical

**Table 1** Baseline Characteristics of the Patients According to 28-Day Survival

	All (n=30)	Survivors (n=21)	Non-Survivors (n=9)	<i>p</i>
Age, mean $\pm$ SD (year)	64.70 $\pm$ 13.95	65.19 $\pm$ 14.92	63.55 $\pm$ 12.10	0.774
Male, n (%)	21 (0.70)	15 (0.71)	6 (0.67)	0.794
BMI, mean $\pm$ SD (kg/m <sup>2</sup> )	24.01 $\pm$ 3.79	24.13 $\pm$ 4.01	23.74 $\pm$ 3.42	0.804
Source of Infection, n (%)				0.628
Lung	13 (0.43)	8 (0.38)	5 (0.56)	
Abdomen	14 (0.47)	10 (0.48)	4 (0.44)	
Urinary	2 (0.07)	2 (0.10)	0 (0)	
Other	1 (0.03)	1 (0.05)	0 (0)	
Comorbidities, n (%)				
Hypertention	15 (0.50)	11 (0.52)	4 (0.44)	0.690
Coronary heart disease	1 (0.03)	1 (0.05)	0 (0)	0.506
Diabetes	2 (0.07)	1 (0.05)	1 (0.11)	0.523
PCT, median (IQR) (ng/mL)	3.06 (0.93, 10.10)	8.73 (1.43, 16.96)	1.62 (0.57, 3.37)	0.041
CRP, median (IQR) (mg/mL)	165.65 $\pm$ 79.64	174.55 $\pm$ 84.37	144.90 $\pm$ 67.10	0.359
Use of CRRT, n (%)	6 (0.20)	4 (0.19)	2 (0.22)	0.842
Use of vasoactive drugs, n (%)	24 (0.80)	16 (0.76)	8 (0.89)	0.426
Use of MV, n (%)	27 (0.90)	18 (0.86)	9 (1.0)	0.232
SOFA score, mean $\pm$ SD	8.47 $\pm$ 4.35	7.52 $\pm$ 3.76	10.67 $\pm$ 5.05	0.069
APACHE II score, mean $\pm$ SD	21.17 $\pm$ 6.50	20.67 $\pm$ 7.17	22.33 $\pm$ 4.74	0.529

**Notes:** Results are presented as mean  $\pm$  SD for continuous variables with a normal distribution and frequencies for categorical variables. Skewed data is presented as median interquartile range, median (IQR).

**Abbreviations:** BMI, body mass index; PCT, procalcitonin; CRP, c-reactive protein; CRRT, continuous renal replacement therapy; MV, mechanical ventilation; APACHE II, acute physiology and chronic health evaluation II; SOFA, sequential organ failure assessment.

ventilation and CRRT, SOFA score or APACHE II score between the survivors and nonsurvivors ( $P > 0.05$  for each). Nonsurvivors had lower levels of PCT [ng/mL, 1.62 (0.57, 3.37) vs 8.73 (1.43, 16.96),  $P=0.041$ ] (Table 1).

## PD-1 Expression on Memory T Cells in Surviving and Nonsurviving Patients with Sepsis

PD-1 expression on naive CD4<sup>+</sup> T cells [%,  $4.99 \pm 0.94$  vs  $4.12 \pm 1.35$ ,  $P=0.617$ ] and naive CD8<sup>+</sup> T cells [%,  $2.05 \pm 0.37$  vs  $3.48 \pm 0.84$ ,  $P=0.079$ ] showed no difference between survivors and nonsurvivors. PD-1 expression on memory CD4<sup>+</sup> T cells in the nonsurvivor group was significantly higher than that in the survivor group [%,  $33.20 \pm 7.08$  vs  $19.10 \pm 2.70$ ,  $P=0.030$ ]. In particular, the percentage of PD-1 on central memory CD4<sup>+</sup> T cells was significantly higher in nonsurvivors than survivors [%,  $33.12 \pm 7.79$  vs  $18.61 \pm 2.73$ ,  $P=0.034$ ], while the percentage of PD-1 on effector memory CD4<sup>+</sup> T cells showed no difference between two groups ( $P=0.177$ ) (Figure 1B). The proportion of PD-1 on memory CD8<sup>+</sup> T cells was significantly increased in nonsurvivors compared to survivors [%,  $44.77 \pm 6.08$  vs  $26.71 \pm 3.00$ ,  $P=0.006$ ]. Both PD-1 expression on central memory CD8<sup>+</sup> T cells [%,  $45.06 \pm 6.88$  vs  $29.97 \pm 3.67$ ,  $P=0.044$ ] and effector memory CD8<sup>+</sup> T cells [%,  $38.82 \pm 4.04$  vs  $22.86 \pm 2.70$ ,  $P=0.003$ ] were increased in patients who died within 28 days after sepsis compared to survivors (Figure 1C).

## The Percentage of PD-1 on Memory T Cells Was Associated with 28-Day Mortality in Sepsis

An ROC curve was subsequently used to evaluate the predictability of the percentage of PD-1 on memory CD4<sup>+</sup> T cells on 28-day mortality in patients with sepsis, which yielded an AuROC of 0.712 (95% CI: 0.518–0.861;  $P=0.033$ ). A cutoff of 21.4% was calculated using the J statistics with a sensitivity of 77.78 and a specificity of 61.90 (Figure 2A). Additionally, an ROC analysis of the percentage of PD-1 on memory CD8<sup>+</sup> T cells on 28-day mortality yielded an AuROC of 0.757 (95% CI: 0.566–0.894;  $P=0.007$ ). A cutoff of 27.5% was calculated, with a sensitivity of 88.89 and a specificity of 61.90 (Figure 2B).

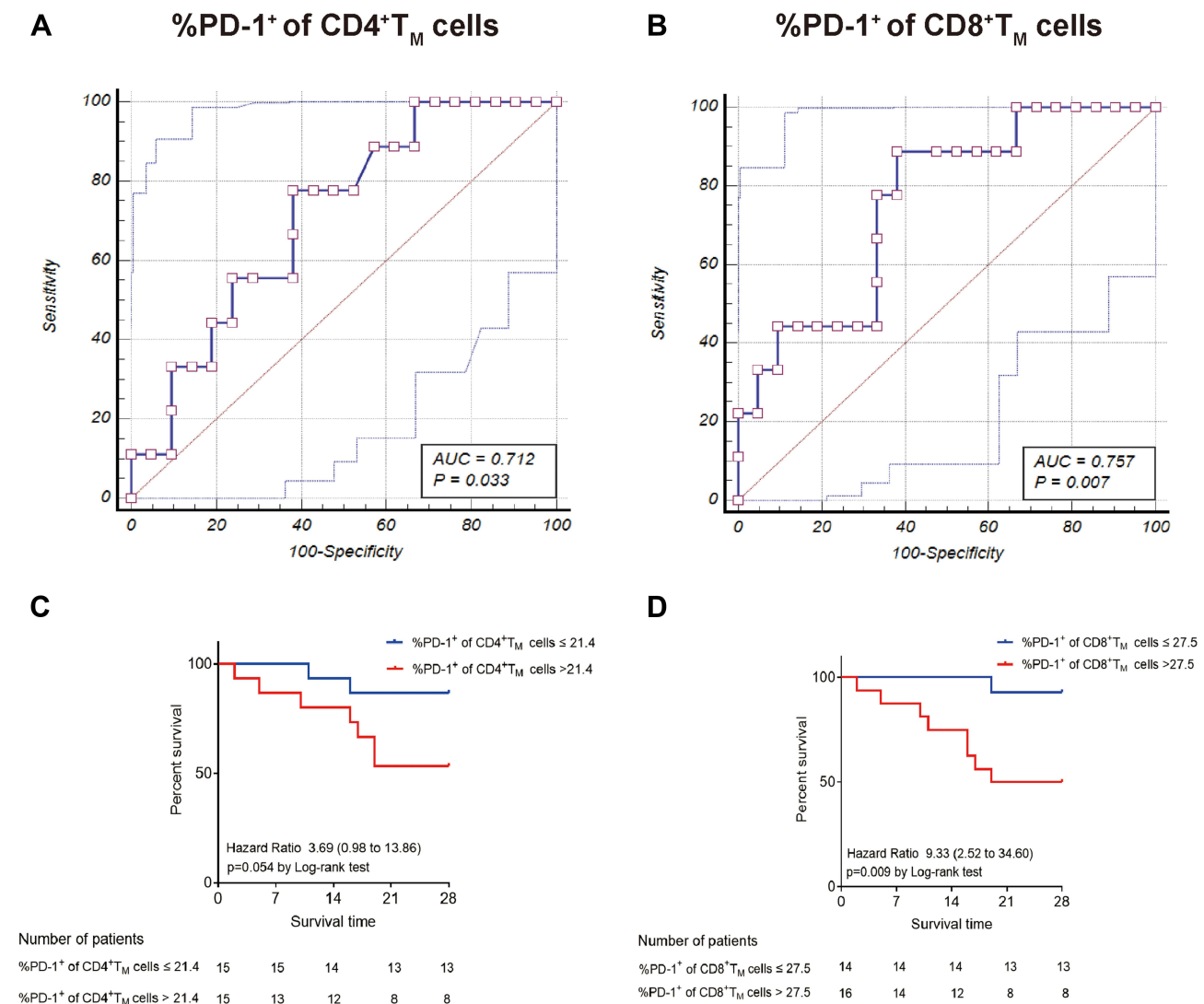
The patients were divided into “high” and “low” PD-1 expression on memory CD4<sup>+</sup> T cells groups according to the optimized 21.4% cutoff level. According to the Log rank test, the 28-day mortality rates between the “high” and “low” groups showed no difference ( $P=0.054$ ), and the Kaplan–Meier curve was elaborated to derive a hazard ratio of 3.69 (95% CI: 0.98–13.86) for percentage of PD-1 on memory CD4<sup>+</sup> T cells regarding 28-day mortality (Figure 2C). The patients were divided into two groups according to the optimized 27.5% cutoff level of percentage of PD-1 on memory CD8<sup>+</sup> T cells for predicting 28-day mortality in septic patients. According to the Log rank test, the 28-day mortality rates between the two groups were significantly different ( $P=0.009$ ), and the Kaplan–Meier curve was also elaborated to derive a hazard ratio of 9.33 (95% CI: 2.52–34.60) for the proportion of PD-1 on memory CD8<sup>+</sup> T cells regarding 28-day mortality (Figure 2D).

Further immunophenotyping of human PD-1<sup>+</sup> memory CD4<sup>+</sup> T cells revealed that these cells had a phenotype with increased CTLA-4 mean fluorescence intensity (MFI) ( $1551.00 \pm 198.90$  vs  $1001.00 \pm 99.58$ ,  $P=0.017$ ), and TIGIT MFI ( $490.80 \pm 18.34$  vs  $371.00 \pm 19.23$ ,  $P<0.001$ ) and decreased CD25 MFI ( $389.80 \pm 58.99$  vs  $632.20 \pm 82.48$ ,  $P=0.020$ ) compared to PD-1<sup>-</sup> memory CD4<sup>+</sup> T cells (Figure 3A). Immunophenotyping of human PD-1<sup>+</sup> memory CD8<sup>+</sup> T cells revealed that these cells had a phenotype with increased TIGIT MFI ( $554.00 \pm 15.23$  vs  $436.80 \pm 19.74$ ,  $P<0.001$ ) and CD28 MFI ( $4959.00 \pm 438.40$  vs  $3495.00 \pm 431.30$ ,  $P=0.021$ ) compared to PD-1<sup>-</sup> memory CD8<sup>+</sup> T cells (Figure 3B). Overall, these results highlighted the important role of PD-1 in memory T cells during sepsis.

## Cytokine Secretion of Memory T Cells in Patients with Sepsis

To determine whether memory T cells exhibit deficiency in cytokine secretion between survivor group and nonsurvivor group, we analyzed the cytokine secretion of IFN- $\gamma$ , IL-2 and TNF- $\alpha$  from memory CD4<sup>+</sup> T cells and memory CD8<sup>+</sup> T cells of septic patients in our study (Figure 4A). We found that the ability of IFN- $\gamma$  secretion of memory CD4<sup>+</sup> T cells was significantly increased in 28-day survivors compared with nonsurvivors [%,  $16.51 \pm 1.84$  vs  $9.59 \pm 2.69$ ,  $P=0.046$ ]. However, IL-2 and TNF- $\alpha$  secretion by memory CD4<sup>+</sup> T cells showed no difference between the two groups (Figure 4B).





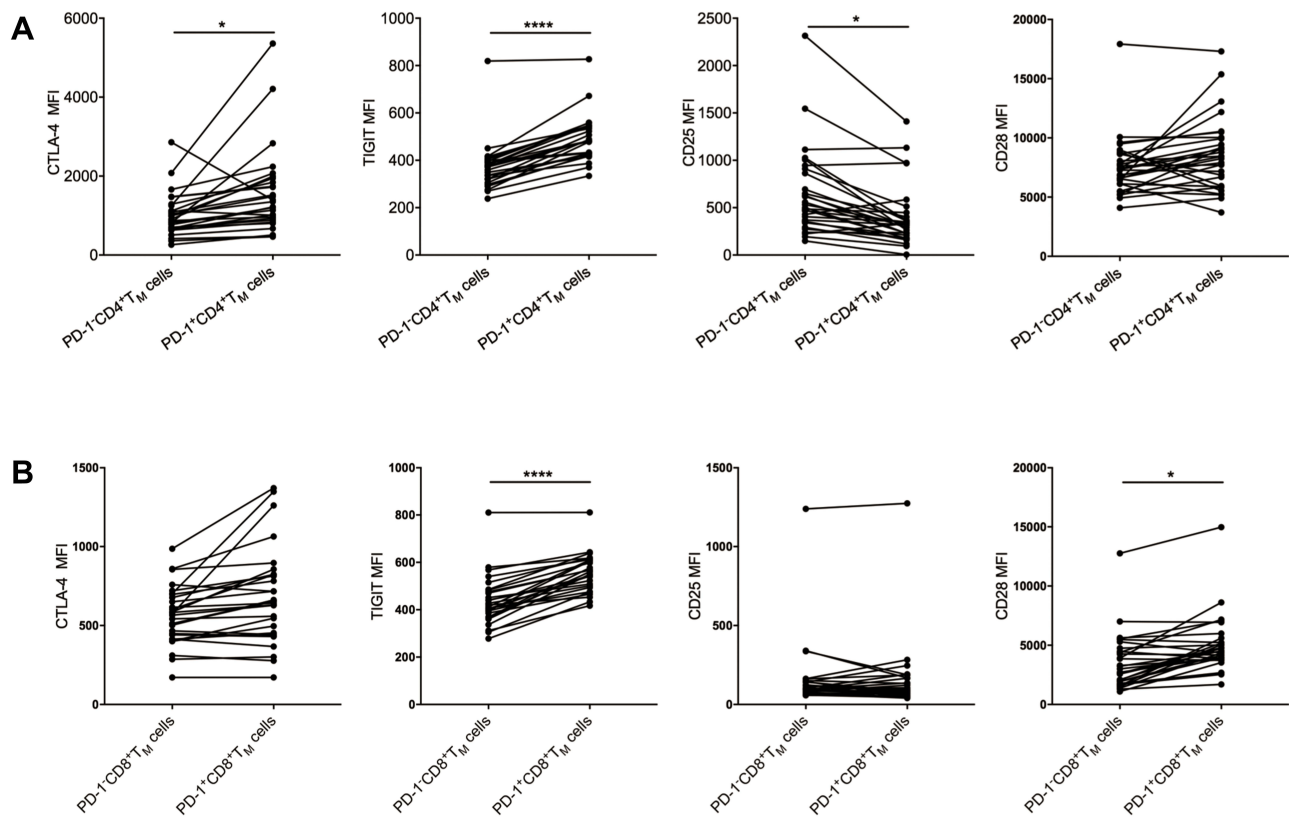
**Figure 2** The expression of PD-1 on memory T cells predicts 28-day mortality during sepsis. (**A** and **B**) ROC analysis of the percentage of PD-1<sup>+</sup> on memory CD4<sup>+</sup> T cells and memory CD8<sup>+</sup> T cells predicting 28-day mortality in septic patients. (**C**) Kaplan-Meier analysis of survival probability in septic patients with PD-1<sup>+</sup> of CD4<sup>+</sup> T<sub>M</sub> >21.4% vs ≤21.4%. (**D**) Kaplan-Meier analysis of survival probability in septic patients with PD-1<sup>+</sup> of CD8<sup>+</sup> T<sub>M</sub> >27.5% vs ≤27.5%.

In 28-day survivors, there was a significant increase in the ability of IL-2 secretion by memory CD8<sup>+</sup> T cells [%; 23.83 ± 3.23 vs 10.34 ± 1.90,  $P=0.014$ ], while IFN- $\gamma$  and TNF- $\alpha$  secretion by memory CD8<sup>+</sup> T cells showed no difference between the two groups (Figure 4C).

## Discussion

In this study, we evaluated the expression of PD-1 on naive and memory T cells in a cohort of patients with sepsis, and found that PD-1 on memory CD4<sup>+</sup> T cells and CD8<sup>+</sup> T cells was significantly elevated in 28-day nonsurvivors in comparison with 28-day survivors, while PD-1 expression showed no difference between survivors and nonsurvivors on naive CD4<sup>+</sup> T cells and naive CD8<sup>+</sup> T cells. The cytokine secretion of memory T cells was significantly decreased in nonsurvivors.

Growing evidence suggests that alterations in the T-cell repertoire can lead to dysfunction of the immune system during sepsis.<sup>20,21</sup> Long-term immunity is provided by memory T cells, and damage to memory T cells resulting from sepsis can make patients susceptible to reinfection. In a preclinical study, Serbanescu and Xie found that there were



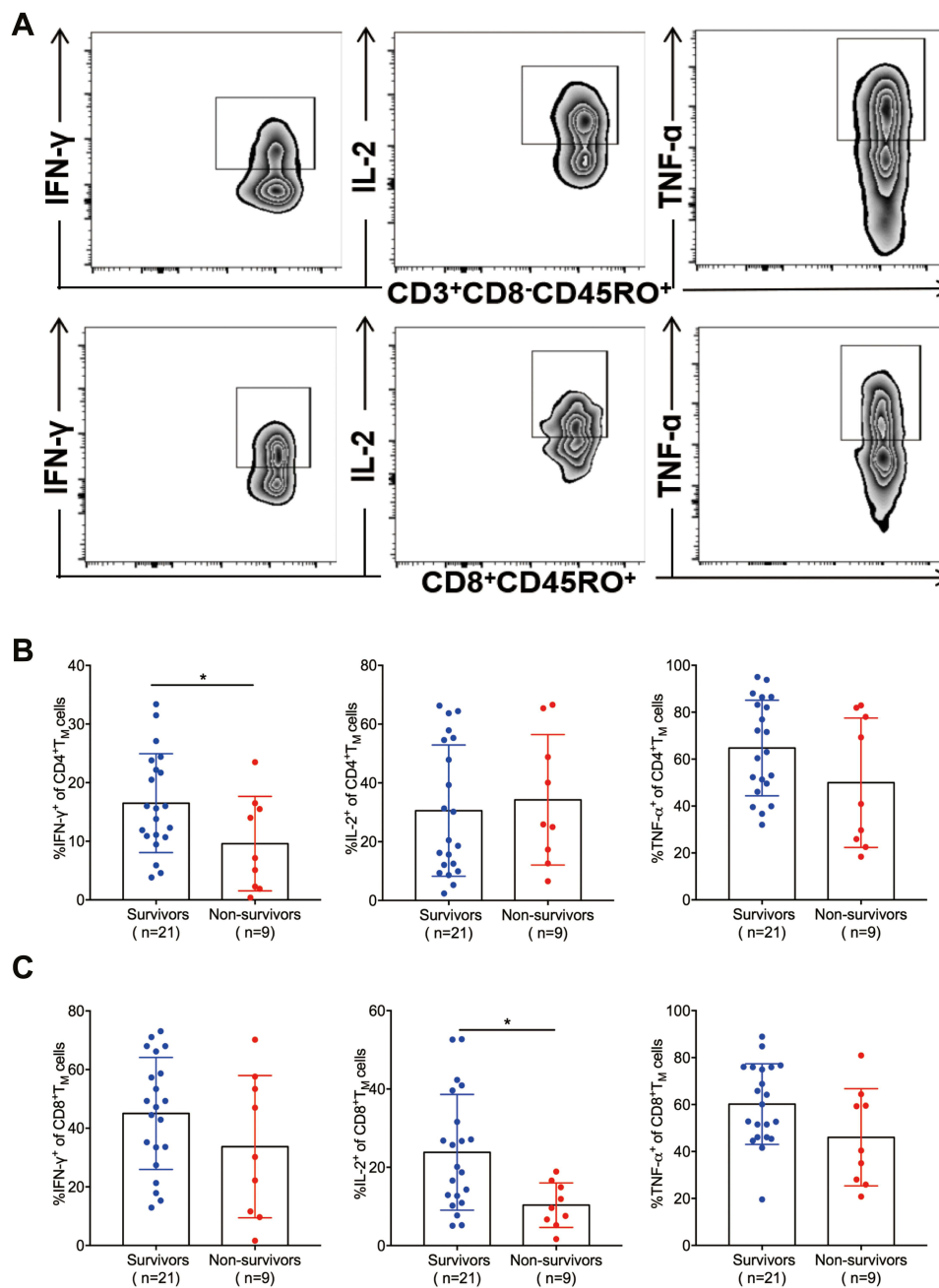
**Figure 3** CD28, CD25, CTLA-4 and TIGIT MFI of PD-1<sup>-</sup> and PD-1<sup>+</sup> memory T cells from septic patients. **(A)** CD28 MFI, CD25 MFI, CTLA-4 MFI and TIGIT MFI of PD-1<sup>-</sup>CD4<sup>+</sup>T<sub>M</sub> cells and PD-1<sup>+</sup>CD4<sup>+</sup>T<sub>M</sub> cells. **(B)** CD28 MFI, CD25 MFI, CTLA-4 MFI and TIGIT MFI of PD-1<sup>-</sup>CD8<sup>+</sup>T<sub>M</sub> cells and PD-1<sup>+</sup>CD8<sup>+</sup>T<sub>M</sub> cells. \**p* < 0.05, \*\*\*\**p* < 0.0001.

**Abbreviation:** MFI, mean fluorescence intensity.

significant reductions in memory CD4<sup>+</sup> T-cells numbers and memory CD8<sup>+</sup> T-cell numbers following CLP,<sup>10,22</sup> revealing the importance of memory T cells in the pathogenesis of sepsis.

The balance of coinhibitory and costimulatory molecules is critical to determine T-cell function during infection. Numerous studies have proven that coinhibitory markers such as PD-1 are highly expressed on T cells, and are associated with T-cell apoptosis during sepsis.<sup>23–25</sup> That anti-PD-1/PD-L1 has been shown to improve survival in cecal ligation and puncture (CLP) model mice and has entered clinical trials in patients with sepsis.<sup>26–28</sup> Our study first reported that it is memory CD4<sup>+</sup> T cells and memory CD8<sup>+</sup> T cells, rather than naive CD4<sup>+</sup> T cells and naive CD8<sup>+</sup> T cells, that played critical role in PD-1 mediating signaling in sepsis. This was based on the following findings: the percentage of PD-1 on naive CD4<sup>+</sup> T cells and CD8<sup>+</sup> T cells between survivors and nonsurvivors showed no difference; however, PD-1 expression on memory CD4<sup>+</sup> T cells and CD8<sup>+</sup> T cells of sepsis patients who died within 28 days were significantly higher than that of patients survived. And proportion of PD-1 on memory CD8<sup>+</sup> T cells can serve as a prognostic marker for patients with sepsis as defined by the Sepsis-3 criteria. Because the area under the ROC of PD-1<sup>+</sup> memory CD8<sup>+</sup> T cells on 28-day mortality of septic patients was 0.757, the 28-day mortality was higher and the survival time was shorter in patients with a higher percentage of PD-1 on memory CD8<sup>+</sup> T lymphocytes.

Memory T cells can be further divided into central memory T cells and effector memory T cells, two subsets with different characteristics. We found that PD-1 expression was increased on central memory CD4<sup>+</sup> T cells in nonsurvivors compared to survivors, while it showed no difference on effector memory CD4<sup>+</sup> T cells. PD-1 expression on central memory and effector memory CD8<sup>+</sup> T cells was different from that on CD4<sup>+</sup> T cells, as it increased both on central memory and effector memory CD8<sup>+</sup> T cells between the two groups. Different subsets of memory T cells play different roles, and subdivision of these cells can help us to identify the cell subpopulation that matters.



**Figure 4** Functional characterization of memory T cells in patients with sepsis. **(A)** Representative levels of the cytokines IFN- $\gamma$ , IL-2 and TNF- $\alpha$  secreted by memory CD3<sup>+</sup>CD8<sup>-</sup> T cells and memory CD8<sup>+</sup> T cells. **(B)** IFN- $\gamma$ , IL-2 and TNF- $\alpha$  secretion of memory CD3<sup>+</sup>CD8<sup>-</sup> T cells between survivors and nonsurvivors. **(C)** IFN- $\gamma$ , IL-2 and TNF- $\alpha$  secretion of memory CD8<sup>+</sup> T cells between survivors and nonsurvivors. \* $p < 0.05$ .

Our study first analyzed the immune phenotypes of PD-1<sup>+</sup> memory T cells and PD-1<sup>-</sup> memory T cells. We found that human PD-1<sup>+</sup> memory CD4<sup>+</sup> T cells showed an exhausted phenotype with increased TIGIT MFI, CTLA-4 MFI and decreased CD25 MFI versus PD-1<sup>-</sup> memory CD4<sup>+</sup> T cells. And PD-1<sup>+</sup> memory CD8<sup>+</sup> T cells exhibited increased TIGIT MFI and CD28 MFI compared to PD-1<sup>-</sup> memory CD8<sup>+</sup> T cells. Understanding the impact of PD-1 signaling on a subset of memory T cells remains an important goal since memory T cells are more susceptible than naive T cells to sepsis induced attrition.<sup>10</sup>

Multiple cytokines, including IFN- $\gamma$ , IL-2 and TNF- $\alpha$ , have been identified as key regulators in T-cell activation, with the ability to trigger T-cell activation response in a TCR-independent manner.<sup>29</sup> Studies have revealed that the



transcriptional profile and chromatin accessibility are altered in memory T cells from sepsis survivors, leading to decreased T-cell cytokine secretion and reduced infection control,<sup>30</sup> which is consistent with our results. We found that the level of IL-2 released by memory CD8<sup>+</sup> T cells and the level of IFN- $\gamma$  released by memory CD4<sup>+</sup> T cells of nonsurvivors were significantly decreased, which may abrogate the response of T cells to infection.

This clinical study also had several limitations. This was a single-center prospective and observational study, and the sample size of this study was limited, which led to false-positive results. Additionally, the proportion of PD-1 on memory T cells was measured at one time point rather than being consecutively monitored.

## Conclusion

Increased expression of PD-1 on memory T cells was associated with 28-day mortality in patients with sepsis. Moreover, in nonsurvivors, the cytokine secretion of memory T cells was reduced.

## Data Sharing Statement

All the data are illustrated in the figures and in the [Supplementary Data](#).

## Ethics Approval

The study was approved by the Zhongda Hospital affiliated to Southeast University Ethics Committee (Southeast University, Nanjing, China, 2020ZDSYLL041-Y01). Informed consent was obtained from each patient or their legal representative prior to enrollment in the study.

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## Disclosure

The authors report no conflicts of interest in this work.

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