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Article

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Early PD-1 and CTLA-4 Expression on Peripheral Blood T Cells and Subsequent Chronic Critical Illness and 28-Day Mortality: A Prospective Cohort Study

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Abstract

Background: Chronic critical illness (CCI) is characterized by persistent organ dysfunction and poor outcomes, yet reliable early biomarkers remain limited. We investigated whether early expression of programmed cell death protein 1 (PD-1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) on peripheral T cells was associated with subsequent CCI and 28-day mortality.

Methods: This prospective observational cohort study included critically ill adults consecutively admitted to the intensive care unit of the First Affiliated Hospital of Shihezi University between July 2025 and July 2026. Within 24 h of ICU admission, clinical and laboratory variables, echocardiographic parameters, circulating cytokines, and PD-1/CTLA-4 expression on T-cell subsets were assessed. Spearman correlation with false discovery rate (FDR) correction was used to examine associations between immune checkpoint expression and cardiac parameters. Receiver operating characteristic analysis evaluated discrimination for subsequent CCI, and Cox

proportional hazards models examined associations with 28-day mortality.

Results: Of 81 patients analyzed, 39 developed CCI, 41 did not, and one died before CCI status could be determined. Patients who developed CCI had higher APACHE II scores, lactate, myocardial injury markers, inflammatory cytokines, and proportions of CD4⁺PD-1⁺, CD4⁺CTLA-4⁺, and CD8⁺PD-1⁺ T cells, but lower albumin and left ventricular ejection fraction (LVEF) (all $P < 0.05$). CD4⁺PD-1⁺ T-cell proportion showed the strongest individual discrimination for CCI (area under the curve [AUC], 0.934; 95% CI, 0.885-0.983). An exploratory model combining CD4⁺PD-1⁺, CD4⁺CTLA-4⁺, and APACHE II score yielded an AUC of 0.977 (95% CI, 0.953-1.000), with 94.87% sensitivity and 87.80% specificity. In the CCI group, CD4⁺PD-1⁺ proportion correlated with left ventricular end-diastolic volume before, but not after, FDR correction ($p = 0.441$, $P < 0.01$; $q = 0.080$). Multivariable Cox analysis identified higher CD4⁺PD-1⁺ T-cell proportion and lower LVEF as independently associated with increased 28-day mortality.

Conclusions: Early increases in PD-1 and CTLA-4 expression on selected T-cell subsets were associated with subsequent CCI. CD4⁺PD-1⁺ expression, particularly when combined with CD4⁺CTLA-4⁺ and APACHE II score, may aid early risk stratification, while its association with mortality warrants further validation.

Trial Registration: The study was approved by the Medical Ethics Committee of the First Affiliated Hospital of Shihezi University (KJ2025-633-01). Clinical trial number: not applicable.

Keywords: Chronic critical illness (CCI); PD-1; CTLA-4; T lymphocytes; cardiac function; intensive care unit (ICU); prospective cohort study

BACKGROUND

Chronic critical illness (CCI) refers to a prolonged condition of critical illness whereby the patients survive the initial injury but do not regain their organ function and continue to require intensive care. According to clinical studies, CCI is generally diagnosed based on the need for at least 14 days' intensive care unit (ICU) stay along with organ dysfunction^[1]. The process of CCI is often associated with constant systemic inflammation, disturbed immune homeostasis, and increased catabolism. Persistent inflammation, immunosuppression, and catabolism (PICS) syndrome is a significant pathological characteristic of CCI and is usually defined by chronic inflammation, immunosuppression, increased protein breakdown, and increased risk of infections^[2-3]. Additionally to these systemic disorders, cardiovascular complications are often seen in critically ill patients, especially in sepsis, and are characterized by myocardial damage or reduced heart function^[4]. However, the relationships between these cardiovascular disorders and the immune changes that occur during CCI remain unclear.

It has become evident that immune checkpoint pathways play an important role in the development of immune dysfunction during critical illnesses. The programmed cell death PD-1 and the CTLA-4 are among the well-known inhibitory receptors found on T cells, and their activation can result in impaired function of T cells^[5-8]. Previous research has shown that increased CTLA-4 expression by CD4⁺ T cells during sepsis is

connected with immune dysfunction and increased 28-day mortality^[7]. Additionally, PD-1/PD-L1 pathway activation was shown to be associated with decreased number of lymphocytes, impaired T cell response, and disease severity^[6,8]. There is support for the participation in prolonged critical illness of the described pathway in the results of the prospective study which showed that higher soluble PD-L1 (sPD-L1) levels in sepsis patients correlated with CCI development in the future^[9]. Collectively, these observations indicate that abnormal regulation of immune checkpoint pathways may contribute to the evolution of acute critical illness into persistent immune dysfunction.

Despite all of the above observations, the association between early changes in PD-1 and CTLA-4 expressions within different T-cell populations and future development of CCI or deterioration of cardiac functions at the same time is still unknown. For this reason, the current study evaluated PD-1 and CTLA-4 expressions in T-cell populations, inflammation biomarkers, and echocardiography parameters during the first 24 hours after patients' admission to the ICU. CCI was defined according to the patients' condition on the 14th day with further follow-up of their condition up to the 28th day. The principal aim was to evaluate the relationships of early T-cell immune checkpoint expression with later CCI development and cardiac functional indices, with additional analyses exploring their associations with short-term clinical prognosis.

1 Materials and Methods

1.1 General Information

The prospective observational cohort study was done at one medical facility, including critically ill adults hospitalized at the Department of Intensive Care Medicine, First Affiliated Hospital of Shihezi University, from July 2025 to July 2026. The total number of patients meeting the inclusion criteria was 87. Six patients died during the first 24 hours following ICU admission and were not included in further analysis, thus there were 81 patients included in the final analysis. During the first day at the ICU, the patients were examined to obtain information about their demographic and clinical status, blood sampling was done to determine the levels of immune checkpoint proteins on T-cell subsets and circulating inflammation markers. Echocardiography was done to assess cardiac structure and function. After enrolment, the patients were divided into the groups based on the criteria of CCI^[10]. Survival and clinical outcomes were then monitored for 28 days from the date of ICU entry.

In case of analyses based on the left ventricular systolic function, the subjects were classified based on their baseline LVEF values into a group with preserved LVEF ($\geq 50\%$) and a group with reduced LVEF ($< 50\%$). The threshold of 50% was selected with regard to the classification criteria established in the 2024 Chinese Guidelines for the Diagnosis and Treatment of Heart Failure^[11]. This division was introduced solely for subgroup comparisons of systolic function and should not be interpreted as a clinical criterion for defining any specific heart failure (HF) phenotype. The design and presentation of this observational investigation followed the recommendations of the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE)

statement. In addition, the exploratory development of the predictive model was reported in accordance with the Transparent Reporting of a Multivariable Prediction Model for Individual Prognosis or Diagnosis combined with Artificial Intelligence (TRIPOD+AI) framework^[12].

This study was conducted in accordance with the Declaration of Helsinki and its later amendments. All experimental protocols involving human participants were reviewed and approved by the Medical Ethics Committee of the First Affiliated Hospital of Shihezi University (Approval No. KJ2025-633-01). All methods were performed in accordance with the relevant institutional guidelines and regulations. Written informed consent was obtained from all participants or their legally authorized representatives prior to enrollment.

1.2 Definition of CCI, Patient Grouping, and Inclusion/Exclusion Criteria

1.2.1 Definition of CCI and Patient Grouping

According to the Research Triangle Institute (RTI) Classification Criteria for ICU Illness (CCI)^[10], CCI was defined as a duration of 14 days or more in the ICU coupled with ongoing dysfunction of one or more organ systems, as shown by the Sequential Organ Failure Assessment (SOFA) score fourteen days after ICU admission. The patients were categorized as Non-CCI if they were in the ICU for less than 14 days without any ongoing organ dysfunction as determined by the SOFA score prior to ICU discharge, or if they were in the ICU for 14 days or more without any ongoing organ dysfunction as determined by the SOFA score on day 14. Patients

who died before day 14 and therefore did not meet the criterion of an ICU stay of ≥ 14 days were classified into the Early death group if persistent dysfunction of at least one organ system was still present according to the SOFA score. Accordingly, all enrolled patients were categorized into three groups: CCI, Non-CCI, and Early death (Figure 1).

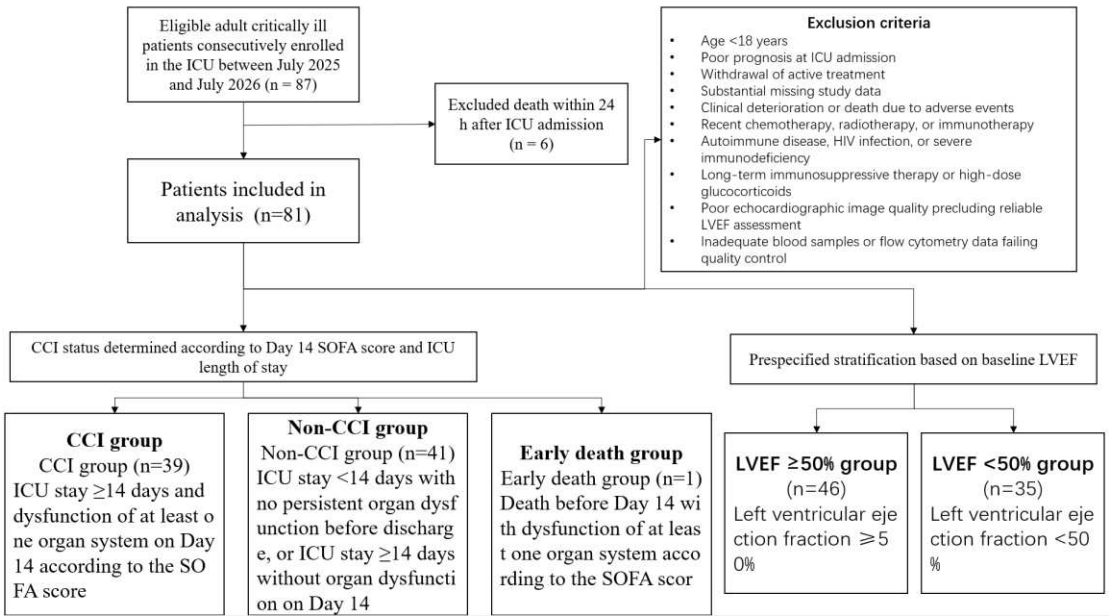


Figure 1. Study flowchart of patient enrollment and group allocation

1.2.2 Inclusion Criteria

(1) age ≥ 18 years; (2) admission to the ICU and fulfillment of the predefined criteria for critical illness; (3) availability of peripheral blood samples and echocardiographic examinations required by the study protocol; (4) complete clinical and 28-day follow-up data; and (5) written informed consent provided by the patient or a legally authorized representative.

1.2.3 Exclusion Criteria

(1) terminal malignancy or end-stage multiple organ failure at ICU admission; (2) withdrawal of active treatment during ICU hospitalization; (3) death within 24 h after ICU admission; (4) recent chemotherapy, radiotherapy, or immunotherapy; (5) autoimmune disease, HIV infection, or other severe immunodeficiency; (6) long-term use of immunosuppressive agents or high-dose glucocorticoids; (7) inadequate echocardiographic image quality for reliable LVEF assessment; or (8) peripheral blood samples or flow cytometry data that did not meet predefined quality-control criteria.

1.3 Main Reagents and Instruments

1.3.1 Main Reagents

ELISA kits for IL-6, IL-1 β , IL-10, and TNF- α (JL14113, JL13662, JL19246, and JL10208, respectively) were obtained from Shanghai Jianglai Biotechnology Co., Ltd. Fluorochrome-conjugated antibodies against CD3, CD4, CD8, PD-1, and CTLA-4 (45-0037-42, 25-0049-42, 406-0088-42, 17-9969-42, and 12-1529-42, respectively) were obtained from Thermo Fisher Scientific. Red blood cell lysis buffer (R1010) and Ficoll lymphocyte separation medium (P8900) were obtained from Beijing Solarbio Science & Technology Co., Ltd.

1.3.2 Main Instruments

The main instruments included a microplate reader (ELx-800, BioTek Instruments, USA), a PowChin flow cytometer (SFLO-4, Pewclin, China), and a low-

speed centrifuge (FL-1042, Anhui Fangling Biomedical Equipment Co., Ltd.).

1.4 Methods

1.4.1 Data Collection

Age, sex, smoking status, alcohol drinking history, comorbidities, and Acute Physiology and Chronic Health Evaluation II (APACHE II) score were among the basic demographic and clinical data obtained from all patients who were included. The following laboratory parameters were measured: lactate, typical blood indices, indicators for renal and nutritional health, liver enzymes, and biomarkers for the heart, such as N-terminal pro-B-type natriuretic peptide (NT-proBNP), cardiac troponin I (cTnI), creatine kinase-MB (CK-MB), lactate dehydrogenase (LDH), and CK. LVEF, fractional shortening (FS), dimensions of the heart chambers, and volumes of the left ventricle were all measures measured by echocardiography. Extra data included duration of stay in the ICU and survival status after 28 days.

1.4.2 Plasma Sample Collection and Inflammatory Cytokine Measurement

Each patient had 5 mL of peripheral venous blood drawn into an EDTA-anticoagulated tube within 24 hours of being admitted to the ICU. After separating the plasma supernatant from the blood samples using centrifugation, it was stored at -80°C until analysis. The levels of IL-6, IL-1 β , IL-10, and tumor necrosis factor- α (TNF- α) in the blood were evaluated using enzyme-linked immunosorbent assay (ELISA) kits. The OD was measured at a 450 nm wavelength.

1.4.3 Flow Cytometric Detection of Immune Cell Expression

The same peripheral blood samples that were EDTA-anticoagulated were used for both flow cytometric analysis and measures of inflammatory cytokines. Once the plasma was separated, peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll density-gradient centrifugation. When necessary, red blood cell lysis buffer was used to extract any residual erythrocytes. In the following step, the cells were washed and resuspended in PBS containing 1% fetal bovine serum (FBS).

PBMCs were incubated in the dark for 30 minutes with fluorescently tagged antibodies against CD3, CD4, CD8, PD-1, and CTLA-4, with 2 μ L of each antibody. Using a PowChin flow cytometer, the cells were resuspended after washing. Before acquisition, quality-control microspheres were used to verify instrument performance and fluorescence stability. Acquisition settings were maintained consistently throughout the study, and fluorescence compensation was performed using single-stained controls. The same quality-control samples and fixed voltage settings were used to minimize inter-batch and inter-day variation.

Every sample was gated using the same protocol. Using FSC-A and SSC-A, lymphocytes were first detected, and then FSC-A and FSC-H were used for singlet selection. CD3⁺ T cells were subsequently gated into CD4⁺ and CD8⁺ subsets, and PD-1 and CTLA-4 expression were evaluated within these subsets. We used negative controls to identify the positive thresholds, and we applied them consistently throughout each batch.

The number of CD3⁺ T-cell events in each sample was at least 10,000. There were 100-5,000 CD4⁺ T-cell events and 100–4,000 CD8⁺ T-cell events included in the analysis. For this data analysis, we flipped to FlowJo version 10.9.0. The percentage of positive cells within the specific CD4⁺ or CD8⁺ T-cell subset was used to express the expression of PD-1 and CTLA-4. No one knew which patients were in which groups, and the analysts had no idea what the clinical outcomes were.

1.4.4 Statistical Analysis

All statistical procedures were carried out in R (version 4.5.2). Continuous data are expressed as mean $\pm$ standard deviation (SD), whereas comparisons between two groups were made with either the two-sample Student's t test or the Mann–Whitney U test, depending on the distributional characteristics of the data. Qualitative variables were described as counts with corresponding proportions [n (%)]. Differences in categorical characteristics were evaluated by Pearson's χ^2 test, with Fisher's exact test used when the assumptions of the χ^2 test were not satisfied. A two-sided P value below 0.05 was regarded as evidence of statistical significance. Levels of significance were indicated by asterisks as follows: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Evaluation of CCI primarily focused on participants whose CCI classification could be determined 14 days after entry into the ICU. Associations between measured parameters were explored with Spearman's rank-based correlation method. Because numerous pairwise comparisons were performed, multiplicity was addressed using the Benjamini-Hochberg adjustment to control the false discovery rate (FDR). For each

association, the correlation coefficient (Spearman's ρ), the original P value, and the FDR-adjusted P value were reported.

The capacity of candidate markers measured during the initial 24 h of intensive care to differentiate patients according to their subsequent CCI outcome was examined through ROC analysis. Both single predictors and combinations of multiple variables were assessed. When several predictors were incorporated into one model, logistic regression was first fitted and the resulting estimated probabilities were subsequently entered into ROC analysis. Model discrimination was characterized by the area under the ROC curve (AUC), its 95% confidence interval (CI), sensitivity, specificity, and the cutoff yielding the best classification performance.

The evaluation of death within 28 days used data from all 81 enrolled participants, including the individual who died before CCI status could be assessed on day 14. Separate ROC analyses were undertaken for the proportions of CD4⁺PD-1⁺, CD4⁺CTLA-4⁺, CD8⁺PD-1⁺, and CD8⁺CTLA-4⁺ T-cell populations. For each immune-cell parameter, the threshold that maximized the combined performance of sensitivity and specificity was selected according to the Youden index.

A landmark approach was adopted for the Kaplan–Meier analysis, with the 14th day after ICU entry defined as the starting landmark. Eighty patients who remained alive on day 14 and had complete outcome information were eligible for this analysis. Their subsequent survival experience was examined from day 14 until day 28. Differences in survival distributions between groups were compared using the log-rank

procedure.

To determine variables related to the risk of death during the 28-day observation period, Cox proportional hazards models were fitted using the entire cohort of 81 patients. Continuous covariates were rescaled according to predefined increments considered clinically interpretable before their inclusion in the models. Associations are expressed as hazard ratios (HRs) accompanied by 95% CIs. Model validity with respect to the proportional hazards requirement was examined using Schoenfeld residual-based diagnostics.

2. Results

2.1 Comparison of Baseline Characteristics Between the CCI and Non-CCI Groups

Patients who developed CCI had higher APACHE II scores and a greater prevalence of chronic cardiac insufficiency, acute exacerbation of chronic cardiac insufficiency, HF, chronic kidney disease (CKD), and atrial fibrillation (AF) than patients who did not develop CCI (all $P < 0.05$). The remaining baseline characteristics did not differ between the two groups (Table 1).

Table 1. Baseline Clinical Characteristics of the CCI and Non-CCI Groups

Variable	CCI group (n=39)	Non-CCI group (n=41)	Test statistic	<i>P</i>
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Age, years	78.00 (63.50-84.00)	79.00 (61.00-85.00)	795.5	0.973
APACHE II score	28.33±4.07	22.37±3.06	7.443	<0.001***
Male sex	22/39 (56.4%)	24/41 (58.5%)	-	1.000
Smoking history	15/39 (38.5%)	16/41 (39.0%)	-	1.000
Alcohol consumption history	6/39 (15.4%)	7/41 (17.1%)	-	1.000
Hypertension	25/39 (64.1%)	31/41 (75.6%)	0.772	0.380
Coronary artery disease	26/39 (66.7%)	27/41 (65.9%)	0	1.000
Chronic cardiac dysfunction	27/39 (69.2%)	15/41 (36.6%)	7.283	0.007**
Acute exacerbation of chronic cardiac dysfunction	17/39 (43.6%)	5/41 (12.2%)	8.369	0.004**
Diabetes mellitus	17/39 (43.6%)	15/41 (36.6%)	0.169	0.681
HF	24/39 (61.5%)	13/41 (31.7%)	6.005	0.014*
CKD	21/39 (53.8%)	9/41 (22.0%)	7.368	0.007**
AF	18/39 (46.2%)	7/41 (17.1%)	6.572	0.010*
Valvular heart disease	6/39 (15.4%)	6/41 (14.6%)	-	1.000
Acute myocardial infarction	6/39 (15.4%)	3/41 (7.3%)	2.280	0.306

Pulmonary hypertension	3/39 (7.7%)	2/41 (4.9 %)	1.615	0.671
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2.2 Comparison of Clinical Characteristics Within 24 h of ICU Admission

During the first 24 h after ICU admission, patients who subsequently developed CCI exhibited lower ALB concentrations. In contrast, the creatinine-to-BUN ratio, GGT, lactate, and WBC levels were significantly elevated in the CCI group (all $P < 0.05$). BUN concentrations also differed between groups ($P < 0.05$), but were higher among patients without CCI. Prealbumin concentrations were comparable between the two groups (Figure 2).

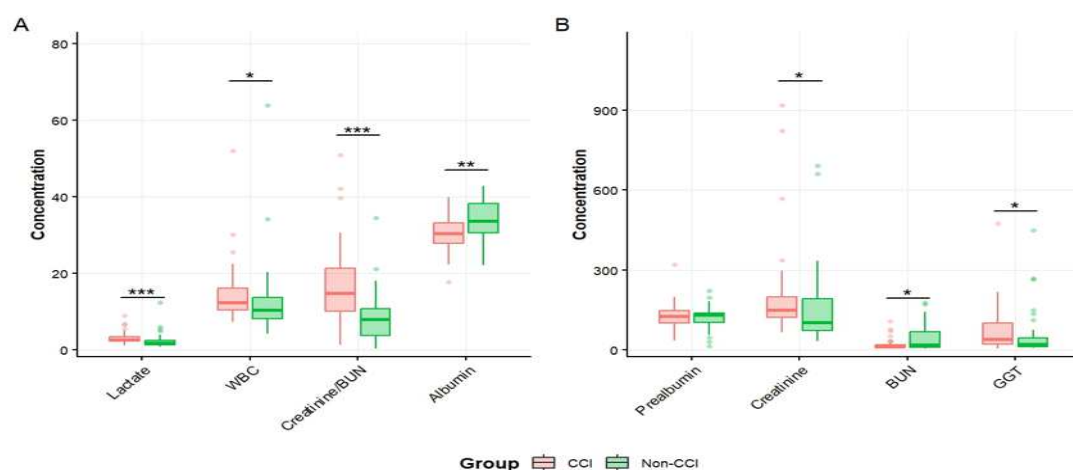


Figure 2. Laboratory parameters measured within 24 h of ICU admission in the CCI and Non-CCI groups

Note: (A) Lactate, WBC, creatinine/BUN ratio, and ALB. (B) GGT, prealbumin, creatinine, and BUN.

2.3 Comparison of Cardiac Function Parameters Between the CCI and Non-CCI Groups

The CCI group had higher CK-MB, cTnI, CK, and LDH (all $P < 0.05$) levels.

LVEF and FS were lower in the CCI group (both $P < 0.05$), whereas left atrial diameter was greater ($P < 0.05$). No significant differences were observed in the other echocardiographic parameters (Figure 3).

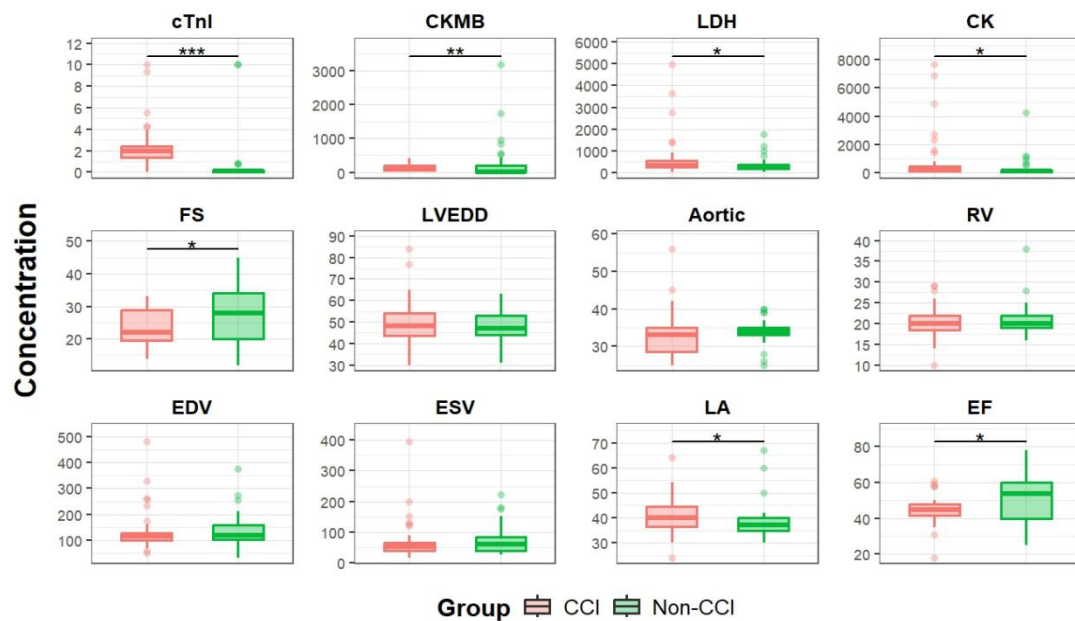


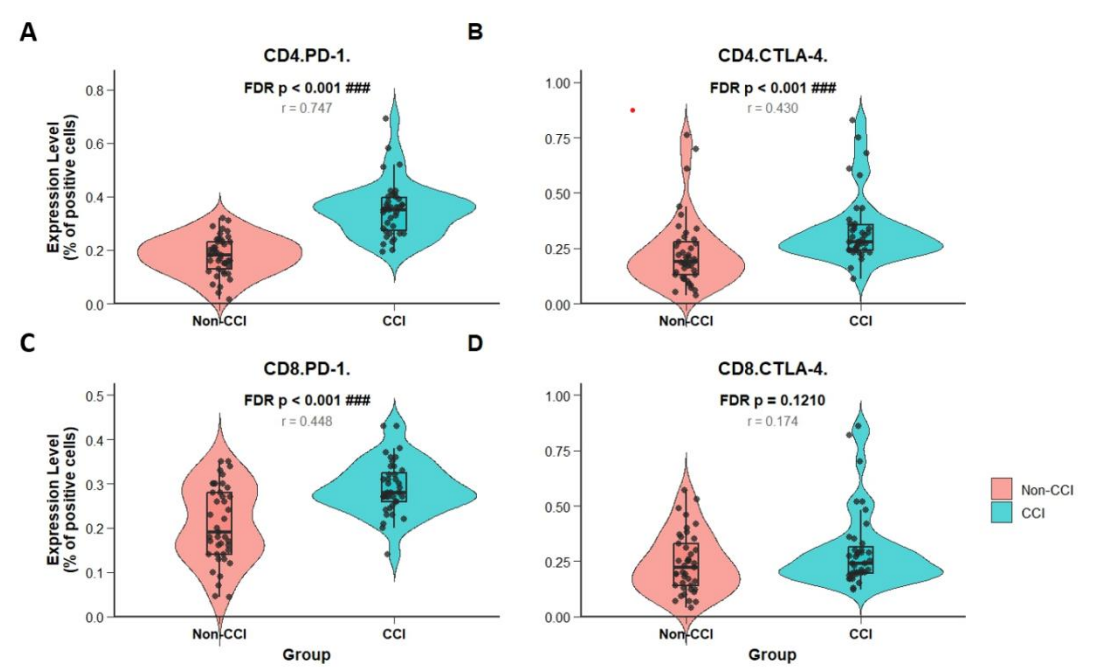
Figure 3. Cardiac function parameters in the CCI and Non-CCI groups within 24 h of ICU admission

Note: Parameters included cTnI, CK-MB, LDH, CK, FS, LVEDD, aortic sinus diameter, right ventricular diameter, EDV, ESV, left atrial diameter, and LVEF.

2.4 Comparison of Peripheral Blood Inflammatory Cytokines and T-Cell PD-1 and CTLA-4 Expression Between the CCI and Non-CCI Groups

Assessment of circulating inflammatory mediators demonstrated markedly increased plasma concentrations of IL-6, IL-10, IL-1 β , and TNF- α in patients who developed CCI (all $P < 0.01$; Figure 4E). Evaluation of immune checkpoint

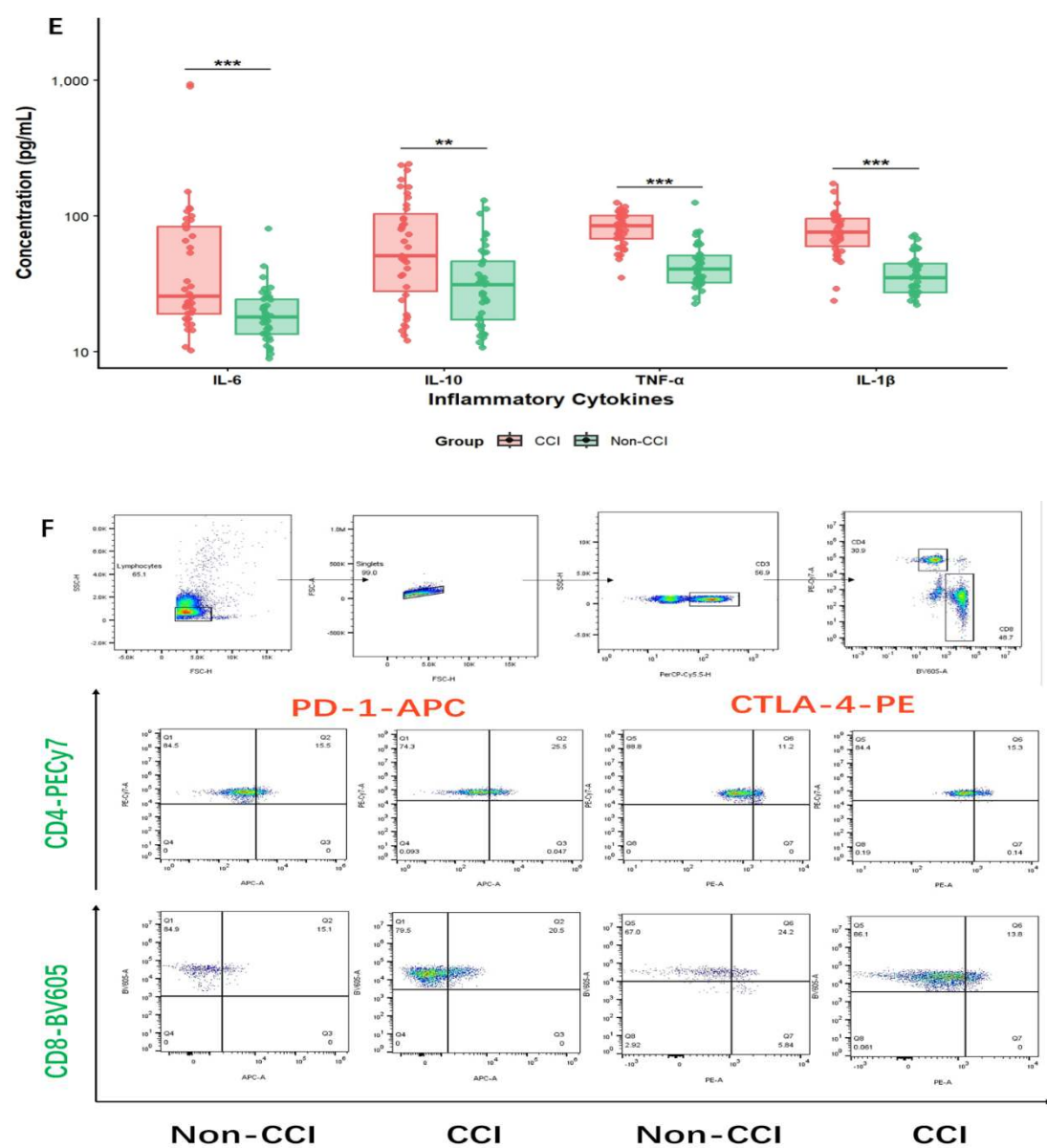
expression on peripheral T-cell subsets likewise revealed greater percentages of CD4⁺PD-1⁺, CD4⁺CTLA-4⁺, and CD8⁺PD-1⁺ T cells in the CCI group (Figure 4A-C). These differences remained significant after adjustment for multiple comparisons using the FDR procedure (all FDR-adjusted P < 0.001). Among these immune parameters, the CD4⁺PD-1⁺ T-cell proportion exhibited the greatest between-group effect size (r = 0.736). By comparison, CD8⁺CTLA-4⁺ T-cell proportions were similar in patients with and without CCI (Figure 4D).



Supplementary Table 2. FDR-Adjusted Statistical Comparisons and Effect Sizes for PD-1 and CTLA-4 Expression on Peripheral Blood T-Cell Subsets Between the CCI and Non-CCI Groups

Parameter	Original P value	FDR-adjusted P value	Effect size (r)
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CD4 ⁺ PD-1 ⁺ T cells	< 0.001	< 0.001	0.747
CD4 ⁺ CTLA-4 ⁺ T	< 0.001	< 0.001	0.430
cells			
CD8 ⁺ PD-1 ⁺ T cells	< 0.001	< 0.001	0.448
CD8 ⁺ CTLA-4 ⁺ T	0.133	0.121	0.174
cells			



* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$; #FDR- $P < 0.05$; ##FDR- $P < 0.01$; ###FDR- P

<0.001.

Figure 4. Comparison of peripheral blood immune checkpoint expression and inflammatory cytokine levels between the CCI and Non-CCI groups

Legend: (A-D) Violin plots showing the proportions of CD4⁺PD-1⁺ (A), CD4⁺CTLA-4⁺ (B), CD8⁺PD-1⁺ (C), and CD8⁺CTLA-4⁺ (D) T cells in the CCI and Non-CCI groups. Between-group comparisons were performed using the Mann-Whitney U test with FDR correction, and effect sizes were estimated using rank-biserial correlation (r). (E) Plasma levels of IL-6, IL-10, TNF- α , and IL-1 β in the two groups. (F) Representative flow cytometric gating strategy for identifying CD3⁺, CD4⁺, and CD8⁺ T-cell subsets and assessing PD-1 and CTLA-4 expression.

2.5 Early Discriminatory Performance of Immune Biomarkers and Exploratory Combined Models for Subsequent CCI

The prognostic discrimination of measurements collected during the first 24 h after entry into the ICU was further evaluated by receiver operating characteristic analysis for subsequent CCI occurrence. When each candidate predictor was considered separately, CD4⁺PD-1⁺ T-cell frequency showed the strongest classification performance, corresponding to an AUC of 0.934 (95% CI, 0.885-0.983). Among the other variables, TNF- α and IL-1 β also demonstrated substantial predictive value, with AUCs of 0.910 (95% CI, 0.843-0.976) and 0.909 (95% CI, 0.841-0.977), respectively. The APACHE II score ranked next, producing an AUC of 0.896 (95% CI, 0.827-0.965). By comparison, the other clinical and immune-related indicators showed only

intermediate ability to differentiate between patients with and without subsequent CCI (Figure 5A).

Combining immune checkpoint markers improved classification beyond that achieved by several single predictors. A model based jointly on the proportions of CD4⁺PD-1⁺ and CD4⁺CTLA-4⁺ cells generated an AUC of 0.945 (95% CI, 0.900-0.990). Further inclusion of the APACHE II score substantially strengthened model discrimination, increasing the AUC to 0.977 (95% CI, 0.953-1.000). However, expanding this model by additionally incorporating the frequencies of CD8⁺PD-1⁺ and CD8⁺CTLA-4⁺ T cells did not lead to a further improvement in AUC (Figure 5B).

At the cutoff identified as optimal, the model comprising CD4⁺PD-1⁺ T-cell frequency, CD4⁺CTLA-4⁺ T-cell frequency, and APACHE II score correctly identified CCI with a sensitivity of 94.87% and a specificity of 87.80%, corresponding to a Youden index of 0.827. After the two CD8⁺ immune checkpoint variables were added, sensitivity decreased slightly to 92.31%, whereas specificity rose to 92.68%, resulting in a Youden index of 0.850. Thus, the expanded five-parameter model did not achieve a higher AUC than the three-parameter model, although the additional CD8⁺ T-cell markers were accompanied by improved specificity and a small gain in the Youden index. Complete performance metrics for the ROC-based evaluations are summarized in Table 2.

Table 2. Apparent Discriminatory Performance of Early Admission Indicators and Exploratory Combined Models for Subsequent CCI Identification

Predictor	AUC	95% CI	Cut-off value	Sensitivity (%)	Specificity (%)	Youden index
APACHE II score	0.896	0.827- 0.965	24.500	87.18	80.49	0.677
Lactate	0.744	0.632- 0.856	2.115	82.05	65.85	0.479
CD4 ⁺ PD-1 ⁺ T cells	0.934	0.885- 0.983	0.245	87.18	82.93	0.701
CD4 ⁺ CTLA-4 ⁺ T cells	0.750	0.638- 0.862	0.225	89.74	63.41	0.532
CD8 ⁺ PD-1 ⁺ T cells	0.760	0.655- 0.866	0.195	97.44	51.22	0.487
IL-6	0.735	0.626- 0.845	29.920	43.59	92.68	0.363
IL-10	0.706	0.590- 0.822	35.587	71.79	68.29	0.401
TNF- α	0.910	0.843- 0.976	51.329	94.87	75.61	0.705
IL-1 β	0.909	0.883-	45.604	94.87	78.05	0.729

			0.984			
CD4 ⁺ PD-1 ⁺ +	0.945	0.900-	0.353	92.31	82.93	0.752
CD4 ⁺ CTLA-4 ⁺		0.990				
APACHE II +	0.977	0.953-	0.363	94.87	87.80	0.827
CD4 ⁺ PD-1 ⁺⁺		1.000				
CD4 ⁺ CTLA-4 ⁺						
APACHE II +	0.977	0.953-	0.449	92.31	92.68	0.850
CD4 ⁺ PD-1 ⁺⁺		1.000				
CD4 ⁺ CTLA-4						
⁺⁺ CD8 ⁺ PD-1						
⁺⁺ CD8 ⁺ CTLA-						
4 ⁺						

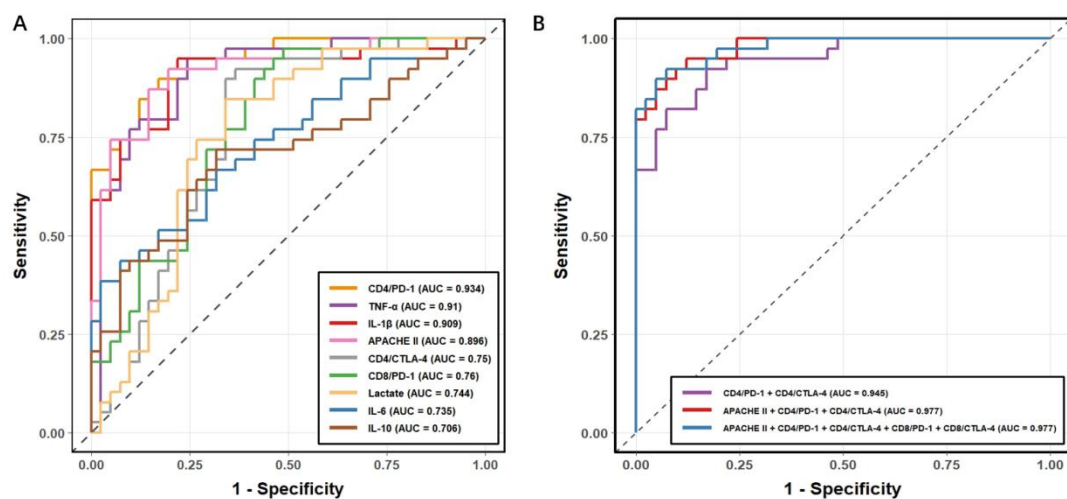


Figure 5. ROC curves of early immune markers, clinical indicators, and exploratory combined models for discriminating subsequent CCI

Note: (A) ROC curves for individual indicators measured within 24 h of ICU admission. (B) ROC curves for exploratory combined models, and the five-factor model.

2.6 Correlations Between Early T-Cell PD-1/CTLA-4 Expression and Cardiac Function-Related Parameters in the CCI Group

Spearman rank correlation analysis was used to examine associations between CD4⁺PD-1⁺, CD4⁺CTLA-4⁺, CD8⁺PD-1⁺, and CD8⁺CTLA-4⁺ T-cell proportions and inflammatory cytokines, echocardiographic parameters, and cardiac function-related laboratory indicators. In the CCI group, CD4⁺PD-1⁺ T-cell proportion was positively correlated with LVEDV before multiple-comparison correction ($\rho = 0.441$, $P < 0.01$), but the association was no longer significant after FDR correction ($q = 0.080$). No significant correlations were observed with LVEF ($\rho = -0.048$, $P = 0.773$, $q = 0.944$), cTnI ($\rho = -0.107$, $P = 0.515$, $q = 0.853$), or NT-proBNP ($\rho = -0.029$, $P = 0.863$, $q = 0.964$) (Figure 6A).

Similarly, in the Non-CCI group, none of the correlations between CD4⁺PD-1⁺ T-cell proportions and LVEDV, LVEF, cTnI, or NT-proBNP reached statistical significance (Figure 6B). Complete results of the Spearman correlation analyses, including the corresponding FDR-adjusted P values, are provided in Supplementary Table S1.

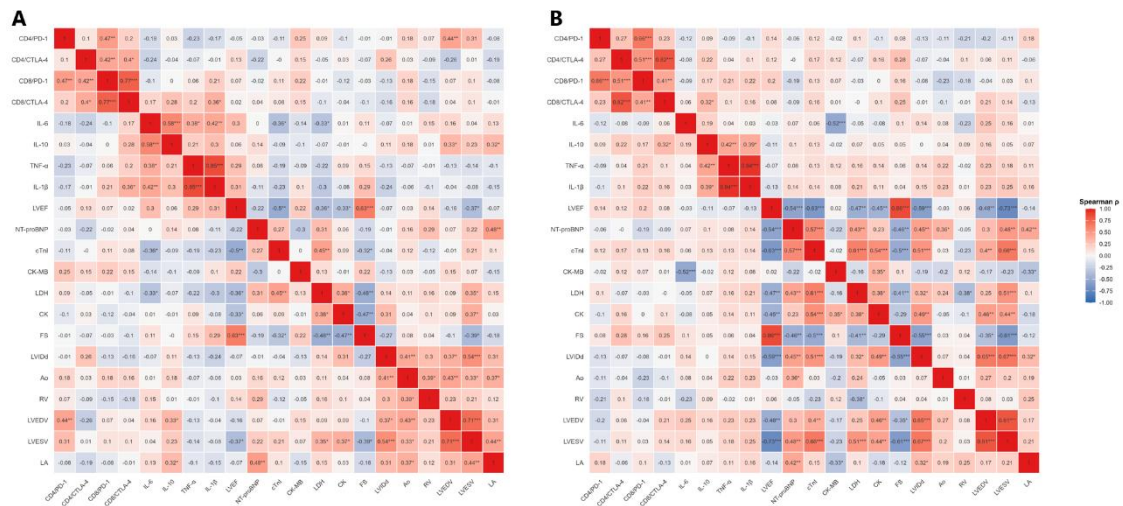


Figure 6. Spearman correlation analysis of early immune checkpoint expression and cardiac function-related parameters in the CCI and Non-CCI groups.

Note: (A) Correlation matrix of CD4⁺PD-1⁺, CD4⁺CTLA-4⁺, CD8⁺PD-1⁺, and CD8⁺CTLA-4⁺ T-cell proportions with inflammatory cytokines, cardiac injury biomarkers, and echocardiographic parameters in the CCI group. (B) Corresponding correlation matrix for the Non-CCI group. Color intensity represents the magnitude and direction of Spearman's ρ , with red indicating positive and blue indicating negative correlations.

2.7 Exploratory Survival Analysis After the Day-14 Landmark

A total of 81 patients were included in the complete cohort. Over the 28-day observation period, 28 deaths occurred, yielding an overall mortality rate of 34.6%. Because one patient died before the day-14 assessment, CCI status could not be assigned for this individual; therefore, analyses based on CCI classification were restricted to the remaining 80 patients. Of the 39 patients who developed CCI, 19 died within 28 days, corresponding to a mortality rate of 48.7%. By comparison, 8 deaths occurred among the 41 patients without CCI, resulting in a mortality rate of 19.5%.

Survival patterns were evaluated according to CCI classification and immune checkpoint expression using Kaplan-Meier analysis, with day 14 after ICU admission designated a priori as the landmark. Between day 14 and day 28, patients with CCI demonstrated a significantly lower cumulative probability of survival than those without CCI (log-rank $P < 0.05$; Figure 7A). A similar analysis based on cardiac function showed that patients with an LVEF below 50% had poorer survival (log-rank $P < 0.05$; Figure 7B).

The ability of the four T-cell checkpoint parameters to differentiate survivors from nonsurvivors at 28 days was further examined by ROC curve analysis. These parameters comprised the frequencies of CD4⁺PD-1⁺, CD4⁺CTLA-4⁺, CD8⁺PD-1⁺, and CD8⁺CTLA-4⁺ T cells. Of these markers, the frequency of CD4⁺PD-1⁺ T cells showed the highest classification accuracy, yielding an AUC of 0.763. A value of 0.295 was identified as the optimal cutoff, corresponding to 75.0% sensitivity, 69.8%

specificity, and a Youden index of 0.448 (Table 3).

A landmark approach was subsequently adopted to evaluate survival after day 14. Participants who remained alive at this landmark were separated into higher- and lower-expression categories using the optimal ROC-based threshold determined for each checkpoint marker. Within the CCI population, patients with higher CD8+PD-1+ levels experienced significantly worse survival between days 14 and 28 than those with lower levels (log-rank $P < 0.01$; Figure 7E). By comparison, survival did not differ significantly when CCI patients were stratified according to CD4+PD-1+, CD4+CTLA-4+, or CD8+CTLA-4+ status (all log-rank $P > 0.05$; Figures 7C, 7D, and 7F). A different pattern was observed among patients without CCI. In this subgroup, increased CD4+PD-1+ expression was accompanied by significantly poorer survival through day 28 (log-rank $P < 0.05$; Figure 7G), whereas categorization according to CD4+CTLA-4+, CD8+PD-1+, or CD8+CTLA-4+ expression yielded no detectable differences in survival (all log-rank $P > 0.05$; Figures 7H-J).

Table 3. ROC analysis of T-cell immune checkpoint markers for predicting

28-day mortality

Predictor	AUC	Optimal cut-off value	Sensitivity (%)	Specificity (%)	Youden index
CD4+PD-1+ T	0.763	0.295	75.0	69.8	0.448

cells

CD4 ⁺ CTLA-	0.620	0.225	85.7	39.6	0.253
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4⁺ T cells

CD8 ⁺ PD-1 ⁺ T	0.671	0.285	71.4	66.0	0.375
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cells

CD8 ⁺ CTLA-	0.547	0.295	82.1	37.7	0.199
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4⁺ T cells

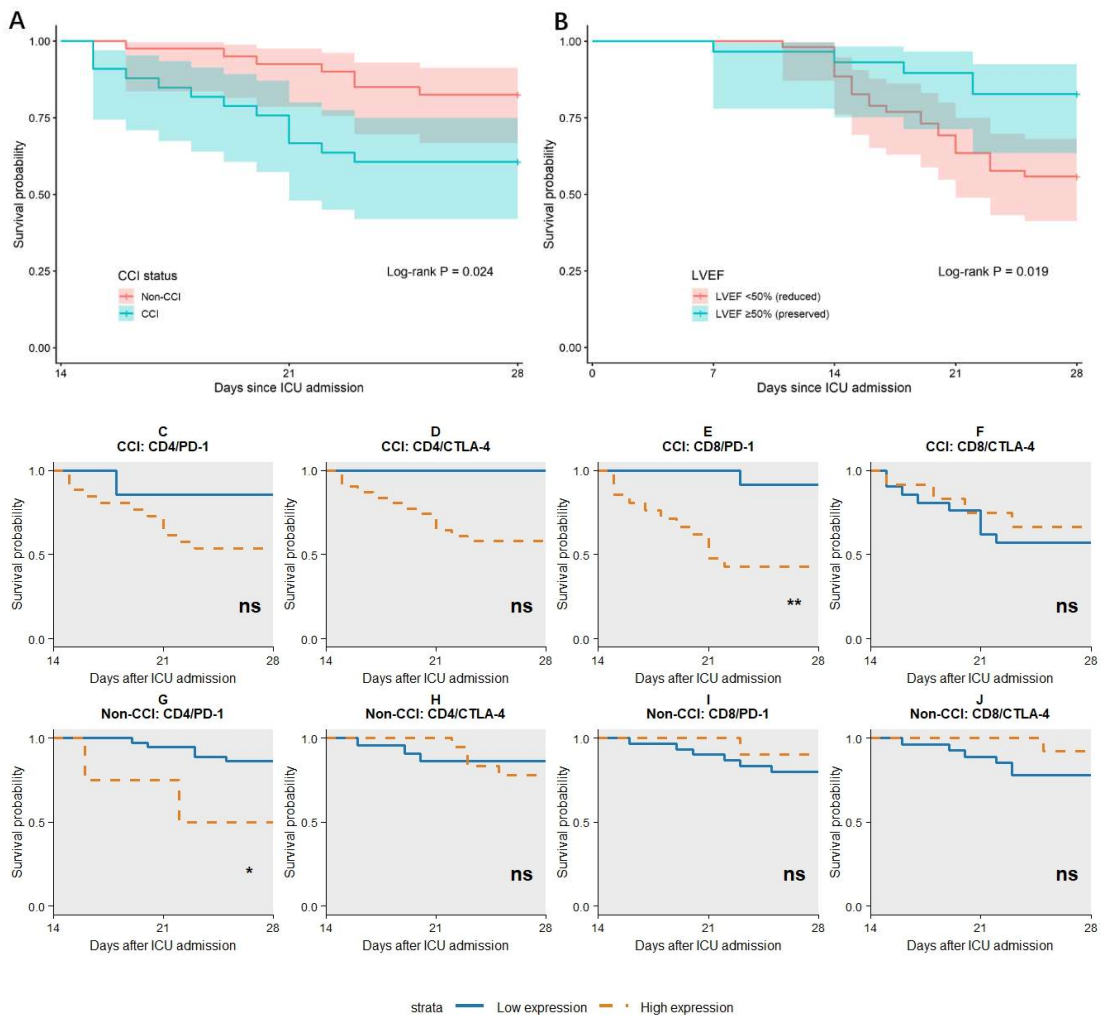


Figure 7. Landmark Kaplan–Meier survival analyses stratified by CCI status,

cardiac function, and T-cell immune checkpoint expression

Legend: (A) Survival from day 14 to day 28 in the CCI and Non-CCI groups. Only patients alive at the day-14 landmark were included; survival was significantly lower in the CCI group ($P = 0.024$). (B) Survival stratified by LVEF, showing lower survival in patients with LVEF $< 50\%$ (log-rank $P = 0.019$). (C-F) Survival in the CCI group stratified by high versus low expression of CD4⁺PD-1⁺ (C), CD4⁺CTLA-4⁺ (D), CD8⁺PD-1⁺ (E), and CD8⁺CTLA-4⁺ (F), based on ROC-derived optimal cutoffs. High CD8⁺PD-1⁺ expression was associated with lower survival ($P < 0.01$), whereas the other markers showed no significant differences. (G-J) Corresponding analyses in the Non-CCI group for CD4⁺PD-1⁺ (G), CD4⁺CTLA-4⁺ (H), CD8⁺PD-1⁺ (I), and CD8⁺CTLA-4⁺ (J). High CD4⁺PD-1⁺ expression was associated with lower survival ($P < 0.05$), whereas the other markers were not significantly associated with survival.

2.8 Exploratory Cox Regression Analysis of 28-Day Mortality in the Entire

Cohort

Potential determinants of death within 28 days were further investigated in exploratory Cox proportional hazards models.

When each candidate factor was assessed individually, a 5-point increase in the APACHE II score corresponded to a higher mortality hazard (HR = 1.477, 95% CI, 1.019-2.140; $P < 0.05$). Similarly, every 10-percentage-point increase in the CD4⁺PD-1⁺ T-cell fraction was associated with an HR of 2.423 (95% CI, 1.707-3.440; $P < 0.001$), while the corresponding increase in CD8⁺PD-1⁺ T cells was associated with an

HR of 1.730 (95% CI, 1.057-2.830; $P < 0.05$). In contrast, greater systolic function was associated with better survival: for each 5-percentage-point rise in LVEF, the mortality hazard decreased to an HR of 0.770 (95% CI, 0.644-0.920; $P < 0.01$). A twofold elevation in IL-1 β was associated with an HR of 1.616 (95% CI, 0.989-2.640; $P = 0.050$), representing only a borderline relationship that did not meet the prespecified threshold for significance. No statistically meaningful associations with 28-day death were observed for age, IL-10, TNF- α , IL-6, CD4⁺CTLA-4⁺ T-cell frequency, or CD4⁺CTLA-4⁺ and CD8⁺CTLA-4⁺ T cells were not significantly related to 28-day mortality (Figure 8A).

A subsequent adjusted Cox model simultaneously included the APACHE II score, LVEF, IL-1 β , and the frequencies of CD4⁺PD-1⁺ and CD8⁺PD-1⁺ T cells. After accounting for the other variables in the model, CD4⁺PD-1⁺ T-cell frequency retained a significant positive relationship with mortality. Specifically, each 10-percentage-point increase was associated with an approximately 2.4-fold greater hazard of death (HR = 2.400, 95% CI, 1.503-3.832; $P < 0.001$). LVEF remained inversely related to mortality, with every 5-percentage-point increase associated with an HR of 0.771 (95% CI, 0.622-0.956; $P < 0.05$). In contrast, the effects observed for the APACHE II score, IL-1 β , and CD8⁺PD-1⁺ T-cell frequency were weakened after covariate adjustment and no longer reached statistical significance (Figure 8B).

Table 4. Univariable and Multivariable Cox Proportional Hazards

Regression Analyses of 28-Day Mortality

Variable	Univariable	P value	Adjusted	P value	PH test P
	HR (95% CI)		HR (95% CI)		
APACHE II score, per 5 points	1.477 (1.019–2.140)	0.039*	1.047 (0.642–1.709)	0.854	0.513
LVEF, per 5 percentage points	0.770 (0.644–0.920)	0.004**	0.771 (0.622–0.956)	0.018	0.166
IL-1 β , per doubling	1.616 (0.989–2.640)	0.05	1.117 (0.576–2.166)	0.734	0.684
CD4 ⁺ PD-1 ⁺ T cells, per 10 percentage points	2.423 (1.707–3.440)	< 0.001***	2.400 (1.503–3.832)	< 0.001	0.823
CD8 ⁺ PD-1 ⁺ T cells, per 10 percentage points	1.730 (1.057–2.830)	0.029*	0.903 (0.479–1.703)	0.754	0.523

points

Note: HRs were estimated using Cox proportional hazards regression and are expressed per 5-point increase in APACHE II score, per 5-percentage-point increase in LVEF, per doubling of IL-1 β concentration, and per 10-percentage-point increase in the proportion of CD4⁺PD-1⁺ or CD8⁺PD-1⁺ T cells.

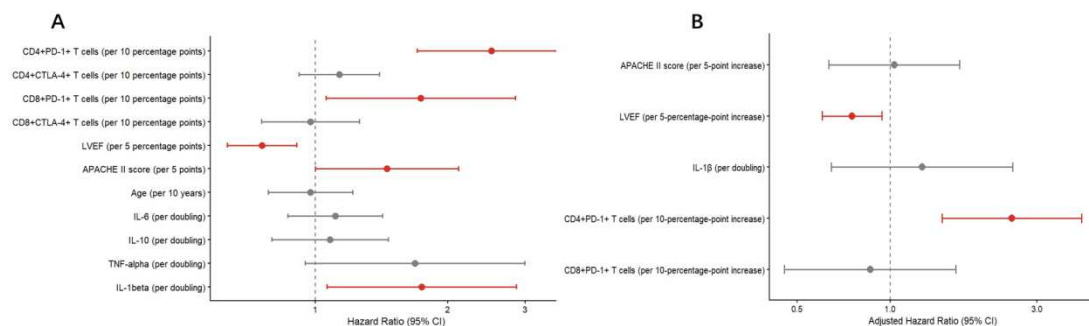


Figure 8. Exploratory Cox proportional hazards regression analyses of factors associated with 28-day mortality in the entire cohort

Note: (A) Univariable Cox regression analyses of clinical characteristics, cardiac function parameters, inflammatory cytokines, and T-cell immune checkpoint markers. (B) Multivariable Cox regression analysis including APACHE II score, LVEF, IL-1 β , CD4⁺PD-1⁺ T-cell proportion, and CD8⁺PD-1⁺ T-cell proportion.

Red indicates statistically significant associations ($P < 0.05$), whereas gray indicates non-significant associations. The dashed vertical line represents $HR = 1.0$. Continuous variables were modeled per 5-point increase in APACHE II score, 5-percentage-point increase in LVEF, doubling of inflammatory cytokine concentrations, and 10-percentage-point increase in T-cell immune checkpoint expression.

Table 5. Schoenfeld Residual Tests of the Proportional Hazards Assumption for the Multivariable Cox Regression Model

Variable	Schoenfeld χ^2	df	<i>P</i>
APACHE II score	0.429	1	0.513
LVEF	1.914	1	0.166
IL-1 β	0.166	1	0.684
CD4 ⁺ PD-1 ⁺ T cells	0.050	1	0.823
CD8 ⁺ PD-1 ⁺ T cells	0.407	1	0.523
GLOBAL	3.926	5	0.560

Note: The proportional hazards assumption was evaluated using Schoenfeld residuals for each covariate and for the model as a whole. The global test was not statistically significant ($\chi^2 = 3.926$, $df = 5$, $P > 0.05$), indicating no evidence that the proportional hazards assumption was violated in the multivariable Cox regression model.

3 Discussion

This study systematically examined the associations of T-cell immune checkpoint expression early after ICU admission with subsequent CCI development, cardiac dysfunction, and short-term clinical outcomes. Patients who subsequently developed CCI exhibited greater expression of PD-1 and CTLA-4 on CD4⁺ T cells

and increased PD-1 expression on CD8⁺ T cells during the early period following ICU admission. These immunologic alterations were coupled with signs of enhanced systemic inflammation, myocardial damage, and poor cardiac function. Of all the immune checkpoint biomarkers analyzed in this study, the PD-1 expression in CD4⁺ T cells was found to be significantly related to cardiac parameters. Additionally, the incorporation of the immune checkpoints into the existing APACHE II scoring system improved the prediction of development of CCI in patients. Furthermore, survival analysis revealed that high percentage of CD4⁺PD-1⁺ T cells and low LVEF were significant determinants of mortality risk within 28 days. Collectively, these findings raise the possibility that upregulation of inhibitory immune checkpoint molecule expression early during ICU admission is an immunologic hallmark of critical illness that predicts CCI development and cardiac dysfunction. It is already known from previous literature that inflammation, immunosuppression, and catabolism are hallmarks of CCI^[2-3]. Expression of inhibitory immune checkpoint molecules, especially PD-1, has been shown previously to affect T-cell responses and increase the risk of infections and poor outcomes. Here, these findings are added to the existing literature, showing that the expression of inhibitory PD-1 immune checkpoints may be part of an immune dysfunction and multiorgan dysfunction interaction in patients developing CCI.

CCI is characterized by persistent organ dysfunction past the stage of acute critical illness and by the necessity of continuous life support in certain patients with

critical illness^[1,13]. Persistent inflammation, immune dysfunction, and dysregulated catabolism are considered central pathophysiological features of this syndrome^[2,3,5]. Immunological profile associated with CCIS probably will not be homogeneous and will depend on the underlying disease and the stage of critical illness. For instance, the recently published article about patients with traumatic injuries described persistent Th17/IL-17 predominance together with inadequate pathogen clearance, instead of uniform immunosuppressive profile^[14]. Analysis of the immune system of the studied group has identified specific profile of CCIS development. The patients who developed CCIS had increased levels of PD-1 and CTLA-4 expression in CD4⁺ T cells and increased percentage of CD8⁺ T cells positive for PD-1. In addition, IL-6, IL-10, IL-1 β , and TNF- α concentrations were increased in the circulation. The coexistence of all these factors indicates that increased immune checkpoint inhibitory activity can develop simultaneously with persistent systemic inflammation during the progression of CCI. This interpretation is broadly compatible with evidence from severe infectious disease, in which activation of the PD-1 axis has been linked to immunosuppressive phenotypes, increased CTLA-4 expression on CD4⁺ T cells has been associated with infection-induced immune dysregulation and unfavorable clinical outcomes, and augmented PD-1/PD-L1 signaling has been related to impaired T-cell responsiveness^[6-8,15]. However, the increased abundance of PD-1- or CTLA-4-expressing T cells observed here should be interpreted cautiously. These changes support the presence of an immune phenotype that may reflect T-cell dysfunction, but they do not, on their own, demonstrate a state of functional T-cell exhaustion. Because

no functional assays of T-cell activity were performed, increased surface expression of either inhibitory receptor cannot be considered sufficient evidence for establishing exhaustion.

Immune alterations following severe infection are also temporally dynamic and involve multiple cellular compartments. In a cohort of 188 patients, nonsurvivors experienced more pronounced decreases in circulating CD4⁺ and CD8⁺ T-cell numbers, reduced T-cell activation, and elevated IL-10 concentrations^[16]. Single-cell studies investigating persistent inflammatory, immunosuppressive, and catabolic phenotypes after severe infection have further revealed substantial heterogeneity involving monocytes, B cells, CD8⁺ T cells, megakaryocytes, and other immune populations^[17]. Similarly, high-dimensional flow cytometric profiling has demonstrated that immune signatures associated with mortality extend beyond T-cell abnormalities to include changes in myeloid-derived suppressor cells and additional leukocyte subsets. Notably, the frequencies of T cells expressing PD-1, CTLA-4, or LAG-3 did not consistently distinguish clinical outcomes across different patient cohorts^[18]. Immune dysfunction may therefore involve multiple inhibitory pathways rather than a single checkpoint axis. Tim-3 signaling, for example, has been implicated in apoptosis of natural killer T cells and unfavorable outcomes during severe infection^[19], whereas experimental evidence indicates that persistent co-upregulation of PD-1, LAG-3, and Tim-3 may accompany sustained T-cell dysfunction after infection. However, the regulatory mechanisms involved may differ

from the canonical T-cell exhaustion programs described in cancer and chronic infection^[20]. These observations underscore the heterogeneity of immune dysfunction in critical illness and support cautious interpretation of individual checkpoint markers.

Additional analyses of immune-inflammatory markers in connection with cardiovascular function metrics yielded a network of interconnected correlations between subjects diagnosed with CCI. Correlations between the expression levels of PD-1 and CTLA-4 in CD4⁺ and CD8⁺ T cell populations generally had a positive correlation, as did correlations between the cytokine markers IL-6, IL-10, TNF- α , and IL-1 β to different extents. Taken together, these results suggest that chronic inflammation and increased immune inhibition might concurrently occur in CCI. Associations among echocardiographic and cardiac injury markers were more consistent with established physiological relationships. LVEF was positively associated with FS, whereas cTnI, NT-proBNP, and several indices of ventricular volume showed inverse correlations with LVEF. In addition, LVEDV and LVESV were strongly and positively correlated, reflecting parallel alterations in ventricular size and systolic function in the setting of cardiac impairment.

Notably, within the CCI subgroup, a higher proportion of CD4⁺PD-1⁺ T cells was associated with greater LVEDV (Spearman $\rho = 0.44$). This relationship, however, differed in both direction and magnitude in patients without CCI, and statistical significance was lost after adjustment for multiple comparisons using the FDR method. Accordingly, this finding should be interpreted cautiously as an exploratory

observation rather than as evidence of a stable or reproducible association. Sepsis-associated cardiomyopathy has been attributed to multiple interacting mechanisms, including systemic inflammation, and metabolic disturbance^[4]. Inflammatory and immunometabolic abnormalities have likewise been implicated in HF and myocardial remodeling^[21]. Against this background, the concurrent immune-inflammatory abnormalities, myocardial injury, and changes in cardiac function observed in the present study may reflect interconnected processes occurring during the progression of critical illness. Nevertheless, the present correlation analyses do not establish a causal relationship between increased PD-1 expression and myocardial injury or cardiac dysfunction.

The present study also assessed the ability of inflammatory cytokines, disease severity measures, and inhibitory T-cell immune checkpoint markers to discriminate subsequent CCI status. Previous prospective research has shown that sPD-L1 measured on day 7 after severe infection has discriminatory value for subsequent CCI development^[9]. The present study provides complementary information by evaluating surface expression of PD-1 and CTLA-4 on specific T-cell subsets early after ICU admission.

Among the individual markers examined, the proportion of CD4⁺PD-1⁺ T cells showed the highest apparent discriminatory performance for subsequent CCI, with an AUC of 0.934. TNF- α and IL-1 β also demonstrated high apparent discrimination, with AUCs of 0.910 and 0.909, respectively, while the APACHE II score yielded an

AUC of 0.896. These findings indicate that early T-cell immune dysregulation, systemic inflammatory activity, and overall disease severity may each provide information associated with subsequent CCI development. Among the immune checkpoint variables evaluated, CD4⁺PD-1⁺ T-cell proportion showed greater apparent discrimination than the other checkpoint markers, suggesting that PD-1 expression on CD4⁺ T cells may be a sensitive indicator of early immune abnormalities in critically ill patients.

Exploratory combined models were subsequently constructed to determine whether integrating PD-1/CTLA-4-related immune phenotypes with conventional measures of disease severity could improve discrimination. The immune-based model combining CD4⁺PD-1⁺ and CD4⁺CTLA-4⁺ T-cell proportions achieved an AUC of 0.945. Addition of the APACHE II score increased the AUC to 0.977, with a sensitivity of 94.87%, specificity of 87.80%, and Youden index of 0.827. Further incorporation of CD8⁺PD-1⁺ and CD8⁺CTLA-4⁺ T-cell proportions produced a five-factor model with the same AUC of 0.977. Although overall discrimination did not improve further, specificity increased to 92.68% and the Youden index to 0.850. These findings suggest that T-cell immune checkpoint profiles and conventional disease severity assessment may capture partly complementary aspects of CCI risk. In this cohort, the combination of CD4⁺ T-cell PD-1 and CTLA-4 expression with the APACHE II score showed high apparent discriminatory performance, whereas addition of CD8⁺ T-cell parameters provided little incremental improvement in AUC,

suggesting some overlap in the information contributed by these variables. From the perspective of model parsimony, the combination of APACHE II score, CD4⁺PD-1⁺ T-cell proportion, and CD4⁺CTLA-4⁺ T-cell proportion may therefore warrant further investigation, while models incorporating additional immune parameters should remain exploratory. It should also be emphasized that all multivariable combinations were derived and assessed using the same study population. The resulting estimates therefore reflect apparent discriminatory performance rather than performance confirmed through external validation. Given the limited sample size, the possibility of model overfitting cannot be ruled out. These models should thus be considered preliminary and hypothesis-generating, and their clinical utility will require confirmation in independent cohorts.

Previous investigations have likewise suggested that increased PD-1 expression on T lymphocytes may be related to poor prognosis in critically ill populations. In a cohort of 180 patients with severe infection, the percentage of PD-1⁺CD3⁺ T cells was independently associated with 30-day mortality. Moreover, adding this immune marker to a prediction model based on APACHE II and SOFA scores improved its ability to distinguish patients at different levels of risk^[22]. Comparable findings have been reported in patients with liver cirrhosis and severe infection, in whom PD-1 expression on CD3⁺, CD4⁺, and CD8⁺ T-cell populations was related to 28-day mortality. Combining these immunological variables with conventional disease-severity indices further enhanced prognostic discrimination^[23]. These observations are

broadly consistent with the present finding that combining immune checkpoint-related parameters with the APACHE II score provided high apparent discriminatory performance, supporting the possibility that immune status may contribute information beyond that captured by conventional assessments of disease severity. Unlike previous studies that primarily examined mortality, the present study used CCI development on day 14 as the clinical endpoint, thereby extending the potential relevance of PD-1/CTLA-4-related biomarkers to persistent critical illness and immune dysfunction.

Research on CCI and persistent inflammation, immunosuppression, and catabolism syndrome (PICS) has additionally highlighted the limitations of relying on measurements obtained at a single time point to represent the dynamic biological course of critical illness. Serial or longitudinal biomarkers may therefore provide complementary information regarding disease evolution. One prospective cohort demonstrated that early temporal alterations in circulating lipoproteins, lipidomic signatures, and gut microbial composition were associated with later development of PICS/PIICS phenotypes^[24]. Data from the multicenter PerCI cohort further indicated that this phenotype was linked to an elevated risk of long-term death and that newly developed functional disability was frequent among survivors^[25]. Subsequent studies have suggested that lactate concentration, lymphocyte count, and SOFA score measured on day 14 may aid in identifying patients with PICS^[26]. In addition, repeated increases in C-reactive protein (CRP) during the first two weeks following

ICU admission have been associated with later PICS development among patients with severe infection^[27]. Together with the present findings, these observations indicate that CCI development likely reflects the interaction of multiple processes, including disease severity, persistent inflammation, and adaptive immune dysfunction, rather than the effect of any single biomarker. Future studies should therefore validate models combining PD-1/CTLA-4 expression with clinical severity measures in independent cohorts and characterize longitudinal changes in immune checkpoint expression. Integration of multidimensional data encompassing immune phenotypes, inflammatory biomarkers, organ function, and clinical severity may facilitate the development of more robust and generalizable models for CCI risk stratification.

In exploratory analyses of 28-day mortality in the entire cohort, multivariable Cox regression showed that a higher proportion of CD4⁺PD-1⁺ T cells and lower LVEF remained independently associated with mortality. These findings indicate that early T-cell immune dysregulation and cardiac functional status at ICU admission may represent distinct immunological and organ-level features associated with short-term outcomes in critically ill patients. Sustained PD-1 overexpression has previously been associated with impaired T-cell function and immune dysregulation^[6,7], suggesting that an increased proportion of CD4⁺PD-1⁺ T cells may serve as a marker of more pronounced immune dysfunction.

Although a greater proportion of CD8⁺PD-1⁺ T cells was related to 28-day mortality in the unadjusted Cox model, this relationship was attenuated and lost

statistical significance after adjustment for other covariates. Such attenuation may arise from shared prognostic information among immune cell subsets or from imprecise effect estimates caused by the relatively small cohort and limited number of deaths. Unlike this scenario, the inverse correlation between LVEF and mortality demonstrates that maintenance of systolic function of the left ventricle can be indicative of high cardiovascular reserve and better ability to withstand the burden of critical illness from a hemodynamic and inflammatory standpoint. Indeed, studies of the predictive value of the APACHE II score in relation to CCI after brain injury have demonstrated that the value of this score may depend on its timing of application, with discrimination being higher closer to the clinical endpoint^[28]. In this particular study, both the APACHE II score and IL-1 β have not shown any statistically significant relationship with mortality when using the adjusted model. These findings should not be taken to mean that there are no relationships between the severity of the disease, inflammation, and prognosis at all; on the contrary, it could be possible that collinearity of the predictors and lack of power due to the limited sample size can explain the above-mentioned findings. Considering the fact that the current research is prospective and single-center, the survival analysis can only be considered exploratory. Confirmation in larger, multicenter cohorts is needed to determine the reproducibility of these associations and to further elucidate their biological and clinical significance.

From the current results, one can conclude that, in patients with organ

dysfunction, increased levels of inflammation and heart failure, measurement of PD-1 levels on T-cells provides additional information about the state of the immune system apart from conventional tools such as the APACHE II scoring system and echocardiography. Therefore, the link between immune dysfunctions in CCI and heart dysfunction should be explored further.

However, there are several issues that must be addressed in relation to the above results. First, it is important to note that the study was carried out on a prospective basis at one institution and had a relatively small sample size. This may have reduced its power and limited its ability to reflect critically ill patients in other environments. In turn, the results of this study cannot be considered to be generally applicable. Second, the surface expression of PD-1 and CTLA-4 on T cells was assessed in this study only once within the first 24 hours of ICU admission. This cross-sectional immune assessment does not capture temporal fluctuations in checkpoint expression as critical illness evolves and therefore cannot define dynamic immune trajectories associated with CCI progression. Third, functional T-cell assays were not performed; consequently, the functional implications of increased immune checkpoint expression could not be directly determined. In addition, the combined models developed in this study were not subjected to internal or external validation, limiting assessment of their stability, reproducibility, and generalizability and restricting further characterization of distinct T-cell immune phenotypes in CCI. Future studies should therefore include larger multicenter cohorts with more heterogeneous patient populations, serially

monitor immune parameters throughout the course of CCI, and incorporate functional assessments of T cells.

4 Conclusions

Elevated early expression of PD-1 on CD4⁺ T cells, CTLA-4 on CD4⁺ T cells, and PD-1 on CD8⁺ T cells during the first 24 h of ICU admission was associated with later development of CCI, suggesting that enhanced inhibitory checkpoint expression may characterize an early T-cell immune phenotype in patients at risk of prolonged critical illness. In exploratory multivariable Cox analysis, a higher proportion of CD4⁺PD-1⁺ T cells remained associated with an increased hazard of 28-day mortality, whereas higher LVEF was associated with a reduced mortality hazard. These observations support further investigation of the interplay between early T-cell immune dysregulation, cardiac function, and clinical outcomes in larger independent cohorts. A model integrating immune checkpoint markers with clinical parameters showed high apparent discriminatory performance in the derivation cohort. These findings warrant independent validation and provide a basis for further investigation of immune dysregulation, cardiac dysfunction, and potential immune-targeted strategies in CCI.

List of abbreviations

AF, atrial fibrillation; ALB, albumin; APACHE II score, Acute Physiology and Chronic Health Evaluation II score; AUC, area under the receiver operating characteristic curve; BUN, blood urea nitrogen; CCI, chronic critical illness; CI,

confidence interval; CK, creatine kinase; CK-MB, creatine kinase-MB; CKD, chronic kidney disease; CRP, C-reactive protein; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; EDTA, ethylenediaminetetraacetic acid; EDV, end-diastolic volume; ELISA, enzyme-linked immunosorbent assay; ESV, end-systolic volume; FBS, fetal bovine serum; FDR, false discovery rate; FS, fractional shortening; GGT, gamma-glutamyl transferase; HF, heart failure; HR, hazard ratio; ICU, intensive care unit; IL, interleukin; LDH, lactate dehydrogenase; LVEF, left ventricular ejection fraction; LVEDD, left ventricular end-diastolic diameter; LVEDV, left ventricular end-diastolic volume; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PBMC, peripheral blood mononuclear cell; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; PICS, persistent inflammation, immunosuppression, and catabolism syndrome; ROC, receiver operating characteristic; SD, standard deviation; SOFA, Sequential Organ Failure Assessment; STROBE, Strengthening the Reporting of Observational Studies in Epidemiology; TNF- α , tumor necrosis factor alpha; TRIPOD+AI, Transparent Reporting of a Multivariable Prediction Model for Individual Prognosis or Diagnosis combined with Artificial Intelligence; WBC, white blood cell.

Declarations

Ethics approval and consent to participate

This study was conducted in accordance with the Declaration of Helsinki and its later amendments. All experimental protocols involving human participants were approved

by the Medical Ethics Committee of the First Affiliated Hospital of Shihezi University (Approval No. KJ2025-633-01). All methods were performed in accordance with the relevant institutional guidelines and regulations. Written informed consent was obtained from all participants or their legally authorized representatives before enrollment.

Consent for publication

Not applicable.

Availability of data and materials

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

Competing interests

The authors declare that they have no competing interests.

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HC conceived the scientific issue addressed in this commentary. TH contributed to conceptualization, methodology, investigation, wrote the original draft and revising the manuscript. JL contributed to investigation. All authors read and approved the final manuscript.

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Clinical trial number

Not applicable.

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