

Lymphocyte Immunosuppression Transcriptomic Score and ICU-Acquired Infection in CAP-Induced Sepsis: A secondary analysis of public transcriptomic cohorts

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Abstract

Background

The transcriptomic basis of susceptibility to ICU-acquired infection (ICU-AI) in sepsis is poorly understood. We examined whether a lymphocyte function gene expression score (LymphoScore) at ICU admission was associated with ICU-AI risk. The MARS cohort ([GEO:GSE65682]; 346 septic ICU patients with ICU-AI annotation) served as the discovery cohort, with ICU-AI defined as infection occurring > 48 h after ICU admission. After hypothesis-free whole-cohort analyses, we tested a pre-specified hypothesis informed by an independent publication in the community-acquired pneumonia (CAP) subgroup (n = 79, 11 events), using parsimonious logistic regression with Firth correction, bootstrap optimism correction, and a LymphoScore-by-pneumonia-type interaction test, followed by endpoint-hierarchy external assessment across [GEO: GSE154918], [GEO: GSE54514], and [GEO: GSE167363].

Results

Whole-cohort analysis was null (minimum adjusted $P = 0.64$; nested cross-validation AUC 0.470). In CAP, IL7R and CD3D were downregulated among patients developing ICU-AI, and LymphoScore was inversely associated with ICU-AI (OR per SD 0.208, $P = 0.012$; Firth OR 0.241; apparent AUC 0.763, bootstrap 95% CI 0.646–0.915; optimism-corrected AUC 0.721), with a significant LymphoScore-by-pneumonia-type interaction ($P = 0.005$; hospital-acquired pneumonia subgroup OR = 1.775). LymphoScore decreased monotonically with disease severity (Spearman $\rho = -0.806$), declined in non-survivors but recovered in survivors (-0.238 vs $+0.160$ /day, $P = 0.048$), was associated with 28-day mortality (OR = 0.627, 95% CI 0.429–0.916, $P = 0.016$), and showed descriptively lower patient-level monocyte HLA-DRA expression in septic patients on single-cell analysis.

Conclusions

Admission lymphocyte transcriptomic suppression was associated with ICU-AI in CAP-induced sepsis, with evidence of etiological heterogeneity supported by multi-layer external evidence. These hypothesis-strengthening findings require prospective validation in ICU-AI endpoint cohorts.

1 Introduction

Sepsis is life-threatening organ dysfunction caused by a dysregulated host response to infection[1], with an estimated 48.9 million cases and 11 million related deaths worldwide in 2017[2], making it one of the leading causes of ICU admission and death in China and globally[3, 4]. With the widespread adoption of early bundled care[5], more patients now survive the acute phase, only to be exposed to a second hit during their ICU stay: the risk of ICU-acquired infection (ICU-AI). The MARS (Molecular Diagnosis and

Risk Stratification of Sepsis) cohort showed that approximately 13.5% of patients with sepsis develop secondary infections in the ICU, with significantly increased attributable mortality[6].

Sepsis-induced immunosuppression is considered a central mechanism underlying susceptibility to ICU-AI[7–10]. Autopsy and prospective studies have demonstrated widespread T-cell exhaustion, lymphopenia, and impaired antigen presentation in patients who die of sepsis[9]; persistently low monocyte HLA-DR expression and lymphocyte counts predict poor outcomes[11, 12] and are associated with the risk of secondary infection[13]. Immunostimulatory therapies targeting this mechanism, such as recombinant IL-7, have shown potential to reverse lymphopenia in clinical trials[14, 15], but identifying the patient subgroups most likely to benefit remains a key bottleneck[16].

Transcriptomics provides a non-invasive tool for assessing host immune status. Blood leukocyte gene expression has been used for sepsis endotype classification[17–19], mortality risk prediction[20], and severity assessment[21]. Notably, however, transcriptomic studies targeting ICU-AI as an endpoint are rare: Bodinier et al.[22] identified a high-risk subgroup for ICU-AI using an 8-gene transcriptomic score (TScore) measured on days 5–7 after ICU admission, but this score relies on a point-of-care multiplex PCR platform and is sampled relatively late; van Vught et al.[6], analyzing the MARS cohort, suggested that the baseline whole-blood transcriptome at ICU admission largely fails to discriminate patients who subsequently develop secondary infection. In 2026, Almontashiri et al.[23] performed a secondary analysis of [GEO: GSE65682] and found significant IL7R downregulation in patients who later developed ICU-AI within the community-acquired pneumonia (CAP)-induced sepsis subgroup, suggesting that pneumonia etiology may modify the association between immunosuppression and ICU-AI; however, that study did not perform multivariable adjustment for the gene-expression association, interaction testing, multiple-testing control, or robustness analyses, and its conclusions were explicitly framed as exploratory by the authors.

Against this background, we adopted a design framework of “hypothesis testing guided by a prior hypothesis” and used four independent public cohorts to systematically evaluate the association between an admission lymphocyte-function transcriptomic score (LymphoScore) and ICU-AI: (1) re-examining the overall predictive capacity of the baseline transcriptome in the whole MARS cohort; (2) treating the CAP-subgroup signal published by Almontashiri et al.[23] as a pre-specified prior hypothesis and assessing whether that same-dataset signal remained robust under penalized regression, interaction testing, and multiple sensitivity analyses; (3) given the absence of a second public cohort with ICU-AI annotation, adopting an endpoint-hierarchy external assessment strategy to assess the biological plausibility and external consistency of the signature across four layers: disease severity gradient ([GEO: GSE154918]), longitudinal dynamics ([GEO: GSE54514]), mortality outcome, and single-cell composition ([GEO: GSE167363]).

2 Materials and methods

2.1 Data sources and cohorts

This is a retrospective secondary analysis based on public databases. All data were obtained from the Gene Expression Omnibus (GEO, <https://www.ncbi.nlm.nih.gov/geo/>) and are de-identified; no additional ethics approval was required. Four datasets were included (Table 1): the discovery cohort [GEO: GSE65682] (the MARS study, a prospective cohort conducted in the ICUs of two tertiary teaching hospitals in the Netherlands[6, 17]; Affymetrix Human Genome U219 Array (GPL13667) platform, whole-blood samples); the dynamic cohort [GEO: GSE54514][24] (Illumina HT-12 v3 platform; longitudinal whole-blood samples collected on days 1–5 after ICU admission); the severity-gradient assessment cohort [GEO: GSE154918][25] (RNA-seq; whole-blood samples from healthy controls and patients with uncomplicated bacterial infection, sepsis, or septic shock); and the single-cell cohort [GEO: GSE167363] [26] (10x Genomics single-cell RNA sequencing of peripheral blood mononuclear cells from septic patients and healthy controls). Raw data were reprocessed through a unified pipeline: sample phenotypes and expression matrices were parsed from GEO series matrix files; probes were mapped to Entrez Gene identifiers using Bioconductor annotation packages (hgu219.db, illuminaHumanv3.db) and converted to official gene symbols via NCBI gene_info, with probes sharing the same symbol averaged. The overall study design and analytical workflow are summarized in Fig. 1.

Table 1
Composition of the study cohorts

Dataset	Role	Platform	Sample composition	Purpose
[GEO: GSE65682] (MARS)	Discovery cohort	Affymetrix Human Genome U219 Array (GPL13667)	346 ICU patients with sepsis (50 ICU-AI); including 79 CAP (11 events) and 65 HAP (9 events)	Primary-endpoint association analysis
[GEO: GSE54514]	Dynamic cohort	Illumina HT-12 v3, whole blood	Longitudinal sepsis samples (≥ 3 time points: 22 survivors, 7 non-survivors)	LymphoScore trajectory slopes
[GEO: GSE154918]	Severity-gradient assessment cohort	RNA-seq, whole blood	40 healthy, 12 uncomplicated infection, 20 sepsis, 19 septic shock	Severity gradient
[GEO: GSE167363]	Single-cell cohort	10x scRNA-seq, PBMC	5 septic patients (10 T0/T6 samples) and 2 healthy controls	Cellular compositional anchoring
Note: ICU-AI was defined as new-onset infection occurring more than 48 h after ICU admission. For [GEO: GSE54514], only patients with at least three longitudinal measurements were included in the trajectory analysis. CAP, community-acquired pneumonia; HAP, hospital-acquired pneumonia; HTA, Human Transcriptome Array; ICU, intensive care unit; ICU-AI, ICU-acquired infection; MARS, Molecular Diagnosis and Risk Stratification of Sepsis; PBMC, peripheral blood mononuclear cell; RNA-seq, RNA sequencing; scRNA-seq, single-cell RNA sequencing.				

2.2 Outcome definitions

The primary endpoint was ICU-acquired infection (ICU-AI), using the original MARS annotation: new-onset infection occurring more than 48 h after ICU admission (the `icu_acquired_infection` field). The comparator group comprised ICU patients with sepsis admitted during the same period who did not develop ICU-AI; healthy controls were not used for primary-endpoint comparisons and served only to illustrate the severity gradient, thereby avoiding the confounded “sepsis versus healthy” comparison. The secondary endpoint was 28-day all-cause mortality.

2.3 Lymphocyte immunosuppression score (LymphoScore)

Based on the sepsis immunosuppression literature[7–15] and prior candidate genes[23], we pre-specified 17 T-cell function-related genes (IL7R, CD3D, CD3E, CD247, LCK, ZAP70, GIMAP1, GIMAP4, GIMAP5, GIMAP6, CCR7, TCF7, CD28, CD2, ITK, SKAP1, and THEMIS), covering IL-7 receptor signaling, TCR complex and signal transduction, thymocyte development, and T-cell homeostasis. Within each dataset, each gene was z-standardized across samples and the resulting values were averaged to construct LymphoScore; lower scores indicate lower lymphocyte function-related transcriptional activity.

2.4 Statistical analysis

Continuous variables were compared between groups using the Mann-Whitney U test; genome-wide differential expression was assessed with Welch's t-test followed by Benjamini-Hochberg false discovery rate (FDR) correction. Whole-cohort predictive capacity was evaluated by elastic-net logistic regression with five-fold nested cross-validation over a 73-gene literature-based prior panel spanning five functional axes (antigen presentation, T cells, MDSC-like cells, immune checkpoints, and monocytes). Based on the prior report and pathway relevance, IL7R, CD3D, and PRKACB were designated as literature-based primary candidate genes, whereas CFLAR and TNFAIP6 were examined as exploratory candidates. Candidate-gene expression was compared between patients with and without ICU-AI using the Mann-Whitney U test. A Bonferroni-adjusted significance threshold of $P < 0.0167$ was applied to the three primary candidates, and nominal P values were reported for exploratory candidates. For the CAP subgroup, associations were estimated by logistic regression: the primary analysis was a pre-specified parsimonious model (LymphoScore plus age; events per variable [EPV] = 5.5), and the full model (additionally adjusted for sex and diabetes mellitus; EPV = 2.75) served as a sensitivity analysis. To correct for small-sample bias with only 11 events, Firth penalized logistic regression (Jeffreys prior) was used to report adjusted ORs with profile penalized-likelihood 95% CIs. Discrimination was reported as the apparent AUC and the 500-replicate bootstrap optimism-corrected AUC; calibration was assessed by the Hosmer-Lemeshow test and quintile calibration curves; clinical utility was evaluated by decision curve analysis (DCA). Robustness analyses included leave-one-out (LOO) analysis and refitting after excluding influential observations (Cook's distance $> 4/n$). Etiological heterogeneity was assessed in the pneumonia cohort ($n = 144$) by fitting an age-adjusted logistic regression model containing LymphoScore, pneumonia type (CAP/HAP), and a LymphoScore-by-pneumonia-type interaction term. The interaction was tested by comparing models with and without the interaction term using a likelihood-ratio test. Survival analysis used the Kaplan-Meier method and the log-rank test. Twenty-eight-day mortality was analyzed as a secondary outcome using logistic regression adjusted for age and sex, with

results reported as odds ratios and 95% confidence intervals. Two-sided $P < 0.05$ was considered statistically significant unless a prespecified multiplicity-adjusted threshold is stated. Analyses were performed in Python 3.12 (pandas, numpy, scipy, statsmodels, and scikit-learn).

2.5 Endpoint-hierarchy external assessment strategy and single-cell analysis

After systematically screening GEO and the literature, we confirmed that, as of September 2026, [GEO: GSE65682] remains the only public whole-blood transcriptomic cohort with ICU-AI (> 48 h) annotation; the REALISM/MIPrea cohort data of Bodinier et al.[22] are not publicly available. We therefore adopted an endpoint-hierarchy external assessment: (1) in [GEO: GSE154918], testing the monotonicity of LymphoScore along the “healthy–uncomplicated infection–sepsis–septic shock” gradient (Spearman correlation); (2) in [GEO: GSE54514], fitting per-patient linear slopes of LymphoScore (requiring at least 3 time points) and comparing survivors with non-survivors; (3) examining the association with 28-day mortality in the whole cohort; and (4) in [GEO: GSE167363], after quality control ($200 \leq$ detected genes < 5000 , mitochondrial fraction $< 15\%$), normalization, ComBat batch correction, PCA, and clustering, annotating cell types by marker genes and summarizing T-cell proportion and monocyte HLA-DRA expression in septic patients and healthy controls. For patient-level summaries, T cells were defined by CD3D expression and monocytes by CD14 or FCGR3A expression. Cell-type proportions and marker expression were calculated from all QC-passed cells in each sample; for UMAP visualization, a maximum of 1,500 QC-passed cells per sample were randomly retained (18,000 cells in total). For septic patients with T0 and T6 samples, corresponding to 0 and 6 h after sepsis recognition, values were averaged within each patient before visualization, and patients were treated as biological replicates. Because only two healthy controls and five septic patients were available, single-cell comparisons were summarized descriptively without inferential testing. We additionally assessed the feasibility of dynamic analysis using MARS paired samples (baseline and at-infection-event).

3 Results

3.1 Cohort characteristics

[GEO: GSE65682] comprised 802 whole-blood samples, of which 346 ICU patients with sepsis had ICU-AI annotation: 50 (14.4%) developed ICU-AI and 296 did not. The pneumonia-induced sepsis subgroup comprised 144 patients: 79 with CAP (11 ICU-AI events, 13.9%) and 65 with HAP (9 events, 13.8%)—a cohort composition identical to that reported by Almontashiri et al.[23], which verifies the reproducibility of our data reprocessing pipeline.

3.2 Whole-cohort analysis

In the whole cohort of 346 patients, no gene passed an FDR of 0.05 between patients with and without ICU-AI (minimum FDR = 0.64; Fig. 2), and nested cross-validation of the 73-gene prior panel yielded an AUC of 0.470 ± 0.036 , indistinguishable from chance. This null result is consistent with the original

analysis of the MARS team[6], suggesting that in an unselected sepsis population mixing etiologies and endotypes, the baseline transcriptome at ICU admission has no ICU-AI predictive capacity; it also provides a “null in the general population” backdrop for the subsequent subgroup hypothesis testing.

3.3 CAP subgroup

Among the three primary candidate genes in the CAP subgroup, IL7R showed significantly lower expression in patients who developed ICU-AI than in those without ICU-AI ($\log_2FC = -0.975$, $P = 0.014$), remaining below the Bonferroni-adjusted threshold for three primary tests ($P < 0.0167$). CD3D showed nominally lower expression ($\log_2FC = -0.743$, $P = 0.033$), and PRKACB showed a downward trend ($\log_2FC = -0.500$, $P = 0.055$). The direction of the IL7R and CD3D findings corroborated the previously reported same-dataset signal[23] (Fig. 3A). The exploratory candidates CFLAR and TNFAIP6 did not show statistically significant differences (nominal $P = 0.073$ and $P = 0.078$, respectively; Supplementary Table S1). Per 1-SD increase in LymphoScore, the OR for ICU-AI was 0.210 (95% CI 0.062–0.715, $P = 0.013$) in the univariable model, 0.208 (0.061–0.706, $P = 0.012$) in the pre-specified parsimonious model adjusted for age, and 0.186 (0.048–0.714, $P = 0.014$) in the full model additionally adjusted for sex and diabetes mellitus; Firth-corrected ORs were 0.238 (0.064–0.689), 0.241 (0.065–0.685), and 0.232 (0.056–0.697), respectively, with direction and significance preserved (Table 2). The parsimonious model achieved an apparent AUC of 0.763 (bootstrap percentile 95% CI 0.646–0.915) and a bootstrap optimism-corrected AUC of 0.721. The univariable model had an apparent AUC of 0.767 (bootstrap percentile 95% CI 0.620–0.899) and a corrected AUC of 0.769, whereas the full model had an apparent AUC of 0.770 (bootstrap percentile 95% CI 0.702–0.931) but a corrected AUC of only 0.676. This divergence suggests that adding covariates aggravates overfitting at this event count and supports the parsimonious model as the primary analysis (Fig. 3B). Calibration showed no evidence of poor fit (Hosmer–Lemeshow $P = 0.904$; Fig. 4B), and decision curve analysis showed positive net benefit across threshold probabilities of approximately 5%–30% (Fig. 4C).

Table 2

Comparison of models for the association between LymphoScore and ICU-AI in the CAP-induced sepsis subgroup (n = 79, 11 events)

Model	Standard logistic OR (95% CI)	P	Firth-corrected OR (95% CI)	Apparent AUC (bootstrap 95% CI)	Optimism-corrected AUC
Univariable	0.210 (0.062–0.715)	0.013	0.238 (0.064–0.689)	0.767 (0.620–0.899)	0.769
Parsimonious model (+ age; primary analysis)	0.208 (0.061–0.706)	0.012	0.241 (0.065–0.685)	0.763 (0.646–0.915)	0.721
Full model (+ age, sex, diabetes)	0.186 (0.048–0.714)	0.014	0.232 (0.056–0.697)	0.770 (0.702–0.931)	0.676

Note: ORs represent the odds of ICU-AI per 1-SD increase in LymphoScore. The univariable model included LymphoScore only; the parsimonious model included LymphoScore and age; and the full model included LymphoScore, age, sex, and diabetes mellitus. Apparent AUCs and their bootstrap percentile 95% CIs were calculated from 500 bootstrap replicates. Optimism-corrected AUCs were obtained by subtracting the mean bootstrap optimism from the apparent AUC and are reported as point estimates. The optimism-corrected AUC may slightly exceed the apparent AUC when the bootstrap-estimated mean optimism is negative, particularly in small samples. The EPV was 5.5 for the parsimonious model and 2.75 for the full model. Calibration of the parsimonious model was assessed using the Hosmer–Lemeshow test ($P = 0.904$). AUC, area under the receiver operating characteristic curve; CAP, community-acquired pneumonia; CI, confidence interval; EPV, events per variable; ICU-AI, ICU-acquired infection; OR, odds ratio; SD, standard deviation.

3.4 Etiological interaction and robustness

In the pneumonia cohort (n = 144), the age-adjusted LymphoScore-by-pneumonia-type interaction was significant (likelihood-ratio $\chi^2=7.91$, $P = 0.005$). Univariable ORs were 0.210 (95% CI 0.062–0.715, $P = 0.013$) in the CAP subgroup, 1.775 (95% CI 0.655–4.810, $P = 0.259$) in the HAP subgroup, and 0.659 (95% CI 0.332–1.306, $P = 0.232$) in the pooled population (Fig. 3C). These results support etiological heterogeneity in the association between LymphoScore and ICU-AI and reduce, but do not eliminate, the possibility of subgroup-selection bias. Regarding robustness, the OR remained below 1 in all 79 LOO iterations (range 0.143–0.244). After excluding the single most influential observation, the OR was 0.185 ($P = 0.012$); after excluding seven observations with Cook's distance $> 4/n$, the OR was 0.009 ($P = 0.014$). Thus, the direction of association was preserved, but the effect estimate was sensitive to influential observations and should be interpreted cautiously.

3.5 Secondary outcome

Among the 321 patients in the whole cohort with mortality follow-up (72 events), each 1-SD increase in LymphoScore was associated with reduced 28-day mortality (OR = 0.627, 95% CI 0.429–0.916, $P = 0.016$, adjusted for age and sex). In the CAP subgroup, 28-day mortality was numerically higher in patients with

ICU-AI than in those without (45.5% [5/11] vs 18.2% [12/66]; Fisher's exact $P = 0.058$; log-rank $P = 0.052$; Fig. 4A), consistent with the prior report[23].

3.6 Endpoint-hierarchy external assessment

(1) Severity gradient: in [GEO: GSE154918], LymphoScore decreased monotonically along the “healthy (0.789)–uncomplicated infection (-0.172)–sepsis (-0.267)–septic shock (-0.930)” gradient (Spearman $\rho = -0.806$, $P = 5.7 \times 10^{-22}$; Fig. 5A), supporting the biological plausibility of a score that decreases with deepening disease severity and immunosuppression. (2) Dynamic trajectories: in [GEO: GSE54514], the mean LymphoScore slope was + 0.160/day in survivors ($n = 22$) versus - 0.238/day in non-survivors ($n = 7$; Mann-Whitney $P = 0.048$; Fig. 5B), suggesting that recovery of lymphocyte functional transcription is associated with favorable outcomes. (3) Cellular compositional anchoring: exploratory patient-level single-cell analysis of [GEO: GSE167363] showed descriptively lower monocyte HLA-DRA expression in septic patients than in healthy controls (mean 0.982 vs 2.524), consistent with an immunosuppressed phenotype[27], and a lower mean patient-level T-cell fraction (0.185 vs 0.312). Given the very small number of biological replicates (5 septic patients and 2 healthy controls), these findings are presented without inferential testing (Fig. 6).

3.7 Feasibility assessment of dynamic analysis using MARS paired samples

Samples annotated as paired sampling in the MARS cohort comprised 16 baseline samples from ICU-AI patients, 8 baseline samples from non-ICU-AI patients, and 3 at-event samples; only 3 patients had both baseline and at-event paired samples, which cannot support a longitudinal pre- versus peri-infection dynamic analysis. This negative feasibility result is reported as is, and dynamic-level assessment was instead performed in the longitudinal [GEO: GSE54514] cohort (see Section 3.6).

4 Discussion

Using a pre-specified hypothesis-testing framework, this study systematically evaluated the association between an admission whole-blood lymphocyte function transcriptomic score and ICU-AI, with three main findings. First, in an unselected ICU sepsis population, the baseline transcriptome could not predict ICU-AI (no genome-wide FDR-significant signal; machine-learning AUC ≈ 0.47), consistent with the original null conclusion of the MARS team[6], suggesting that previous failures to predict secondary infection from baseline may reflect dilution by population heterogeneity rather than insufficient sample size[28]. Second, in the biologically more homogeneous subgroup of CAP-induced sepsis, lower LymphoScore was associated with increased ICU-AI risk after age adjustment; this finding was supported by Firth correction, bootstrap optimism correction, LOO analysis, and an age-adjusted etiological interaction test, although Cook's distance analyses indicated sensitivity to influential observations. Our reanalysis corroborated the prior same-dataset signal (IL7R and CD3D downregulation) published in 2026[23]. Third, although no second public cohort with ICU-AI annotation

exists for direct external validation, LymphoScore showed a consistent evidence chain compatible with “lymphocyte immunosuppression” across four independent layers: severity gradient, longitudinal dynamics, mortality outcome, and single-cell composition.

Compared with the prior study, the incremental value of our work lies in methodological extension. Almutashiri et al.[23] first reported the IL7R signal in the CAP subgroup, but that study did not adjust the gene-expression association for confounders, perform interaction testing, evaluate overfitting, or apply multiple-testing correction, and its transcriptomic findings were explicitly framed as hypothesis-generating. Because both studies analyzed [GEO: GSE65682], this agreement should be interpreted as same-dataset corroboration rather than independent replication. Within our CAP analysis, restricting the primary model to LymphoScore and age increased the EPV from 2.75 in the full model to 5.5, and the parsimonious model yielded an optimism-corrected AUC of 0.721 compared with 0.676 for the full model. Firth regression accounted for small-sample bias, yielding a slightly attenuated OR estimate (0.24 vs 0.21) without changing the direction of association; the age-adjusted interaction test suggested etiological heterogeneity ($P = 0.005$); and LOO showed that the direction of association never reversed across 79 iterations. Together, these analyses provide hypothesis-strengthening evidence supported by multiple robustness checks, while Cook's distance analyses indicate that the magnitude of the effect estimate remains sensitive to influential observations.

The differential effect between CAP and HAP has potential biological explanations. CAP patients present from the community without preceding healthcare exposure, and their immune homeostasis collapses acutely under the infectious insult, such that differences in lymphocyte functional reserve may already be discernible at baseline; in contrast, HAP patients have typically experienced chronic hits such as surgery, hospitalization, or prior infection before admission, and immunosuppression may already be prevalent at baseline with an evident ceiling effect, depriving LymphoScore of discriminatory power. This speculation is compatible with the direction reversal ($OR > 1$) observed in the HAP subgroup but requires prospective validation. The robust downregulation of IL7R, together with the nominal downregulation of CD3D, has potential mechanistic implications: the IL-7/IL-7R axis is a core pathway of T-cell homeostasis and survival[14, 15], and CD3D is an essential component of the TCR signaling complex whose reduced expression is associated with T-cell dysfunction[8, 29]; these signals are compatible with “adaptive immune paralysis” as a candidate mechanism of ICU-AI susceptibility[30] and offer potential molecular markers for patient-enrichment strategies in immunostimulatory therapies such as IL-7[14, 16].

Our endpoint-hierarchy assessment strategy should be interpreted within the practical constraints of the public data ecosystem. Systematic screening confirmed that [GEO: GSE65682] is currently the only public whole-blood transcriptomic cohort with ICU-AI (> 48 h) annotation; although the TScore study of Bodinier et al.[22] validated the concept that “a transcriptomic score can identify patients at high risk of ICU-AI” (their score was measured on days 5–7 after ICU admission, temporally complementary to our admission-baseline assessment), its data are not public and cannot be used for external validation of our signature. We therefore replaced single-cohort validation with an endpoint-hierarchy evidence chain: [GEO: GSE154918] assesses severity monotonicity of the score (biological gradient), [GEO: GSE54514]

assesses whether dynamic recovery of the score is associated with survival (longitudinal plausibility), the whole-cohort mortality analysis evaluates the association with a hard endpoint (clinical relevance), and single-cell data provide exploratory patient-level cellular anchoring of the immunosuppressive signal. Although this strategy is not equivalent to direct external validation of the ICU-AI endpoint, the consistency of evidence across layers reduces the overall probability that the finding is a false positive.

This study has several limitations. First, the CAP subgroup contained only 11 events, and the EPV of 5.5 in the parsimonious model remained below the conventional threshold of 10. We therefore used a pre-specified parsimonious model, Firth correction, bootstrap optimism correction, leave-one-out analysis, and Cook's distance sensitivity analyses; nevertheless, the optimism-corrected AUC of 0.721 indicates only moderate discrimination, and the findings should be regarded as hypothesis-strengthening rather than confirmatory. Second, ICU-AI annotation was available in only one discovery cohort. Although MARS was conducted in two tertiary teaching hospital ICUs, cohort-specific effects and annotation misclassification cannot be excluded because ICU-AI diagnoses relied on the original cohort annotation without independent adjudication. The endpoint-hierarchy assessment across disease severity, longitudinal trajectory, mortality, and single-cell composition supports biological consistency but does not replace direct external validation in another ICU-AI-annotated cohort. Third, despite the significant interaction test, the CAP analysis remains a subgroup analysis, and residual selection bias cannot be fully excluded; the HAP subgroup contained only 9 events and was underpowered to support a definitive null conclusion. Fourth, the LymphoScore panel includes the prior candidate genes IL7R and CD3D, so the score-level and candidate-gene analyses are not fully independent. Fifth, although gene expression was measured at ICU admission, before the protocol-defined onset of ICU-AI, residual confounding by baseline illness severity or incipient secondary infection cannot be excluded, and SOFA or APACHE scores were unavailable for adjustment. Sixth, the single-cell analysis included only 2 healthy controls and 5 septic patients; although longitudinal T0/T6 samples were averaged within each patient, the very small number of biological replicates precludes reliable inferential testing, and no inferential P values were therefore reported. These data should be interpreted as exploratory cellular anchoring. Finally, only 3 MARS patients had paired baseline and infection-onset samples, so dynamic assessment was performed in [GEO: GSE54514] rather than in MARS. Prospective multicenter studies with adjudicated ICU-AI endpoints, illness-severity adjustment, and serial sampling are required to establish the clinical utility of LymphoScore.

In summary, this study provides hypothesis-strengthening evidence, supported by multiple robustness analyses, that low expression of lymphocyte function-related transcripts at ICU admission is associated with ICU-AI risk in patients with CAP-induced sepsis, and the multi-cohort endpoint-hierarchy evidence chain supports its biological plausibility. Future studies should directly validate this signature in multicenter prospective cohorts designed with ICU-AI endpoints and evaluate its incremental predictive value in combination with clinical variables (e.g., mHLA-DR and lymphocyte counts), as well as its potential to guide patient enrichment for immunostimulatory therapy.

5 Conclusions

In an unselected ICU sepsis population, the admission baseline transcriptome did not show predictive capacity for ICU-acquired infection in this cohort; however, in the CAP-induced sepsis subgroup, a reduced LymphoScore was associated with increased ICU-AI risk, with evidence of etiological heterogeneity. LymphoScore was assessed in additional public datasets across four layers: severity gradient, dynamic trajectory, mortality outcome, and cellular composition. The clinical value of this signature as a tool for ICU-AI risk stratification and immunotherapy enrichment awaits prospective validation.

Abbreviations

AUC

area under the receiver operating characteristic curve

CAP

community-acquired pneumonia

CI

confidence interval

DCA

decision curve analysis

EPV

events per variable

FDR

false discovery rate

GEO

Gene Expression Omnibus

HAP

hospital-acquired pneumonia

HLA-DRA

major histocompatibility complex class II DR alpha

ICU

intensive care unit

ICU-AI

ICU-acquired infection

IQR

interquartile range

log₂FC

log₂ fold change

LOO

leave-one-out

MARS

Molecular Diagnosis and Risk Stratification of Sepsis

MDSC
myeloid-derived suppressor cell
OR
odds ratio
PBMC
peripheral blood mononuclear cell
PCA
principal component analysis
QC
quality control
ROC
receiver operating characteristic
scRNA-seq
single-cell RNA sequencing
SD
standard deviation
T0/T6
0 and 6 hours after sepsis recognition
TCR
T-cell receptor
UMAP
uniform manifold approximation and projection.

Declarations

Ethics approval and consent to participate

This study was conducted in accordance with the Declaration of Helsinki. It involved secondary analysis of publicly available, de-identified transcriptomic datasets from the Gene Expression Omnibus (GEO). Ethics approval and informed consent for data collection were obtained by the investigators of the original studies, as described in the respective primary publications. Because all data are fully de-identified and publicly accessible, and no individual participant data beyond those released by the original studies were used, no additional institutional ethics approval or informed consent was required for the present analysis.

Consent for publication

Not applicable.

Availability of data and materials

The datasets analyzed in this study are publicly available in the Gene Expression Omnibus under the following accession numbers: [GEO: GSE65682], [GEO: GSE54514], [GEO: GSE154918], and [GEO: GSE167363]. The processed analysis tables and custom Python code used for data processing, statistical analysis, and figure generation are publicly available at ScienceDB (<https://doi.org/10.57760/sciencedb.016dm>) and at GitHub (<https://github.com/special163/lymphoscore-icuai>).

Competing interests

The authors declare that they have no competing interests.

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Author contributions

Xiao Zhong: Conceptualization, Methodology, Software, Formal analysis, Investigation, Data curation, Writing—original draft, Visualization. Dong-Li Wang: Validation, Resources, Writing—review and editing. Mei Lin: Supervision, Project administration, Writing—review and editing. All authors read and approved the final manuscript.

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Figures

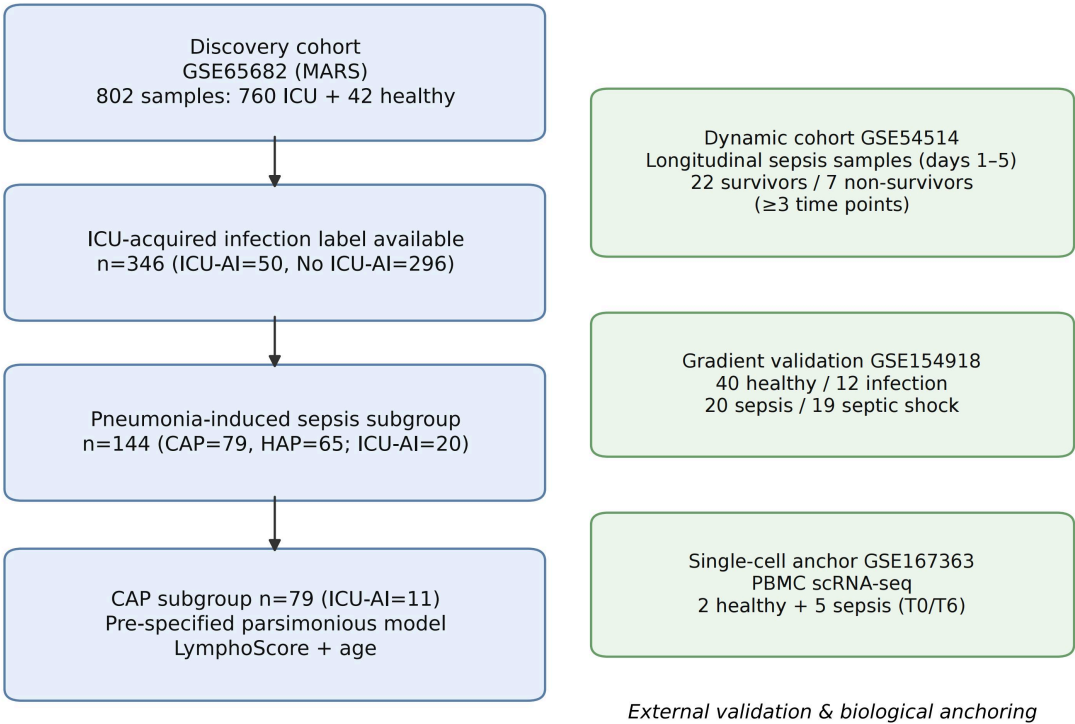


Figure 1

Study design and analytical workflow. The discovery cohort ([GEO: GSE65682], MARS) contained 802 samples, including 760 ICU patients and 42 healthy controls. After screening for ICU-AI annotation, 346 patients with sepsis were included in the primary-endpoint analysis (50 with ICU-AI and 296 without). The pneumonia-induced sepsis subgroup comprised 144 patients (79 CAP and 65 HAP; 20 ICU-AI events), and the pre-specified parsimonious model was evaluated in the CAP subgroup (n=79, 11 events). External assessment used longitudinal samples from [GEO: GSE54514] (22 survivors and 7 non-survivors with ≥ 3 time points), severity-gradient samples from [GEO: GSE154918] (40 healthy controls, 12 uncomplicated infection, 20 sepsis, and 19 septic shock), and PBMC scRNA-seq data from [GEO: GSE167363] (5 sepsis and 2 healthy controls). Abbreviations: CAP, community-acquired pneumonia; HAP, hospital-acquired pneumonia; ICU, intensive care unit; ICU-AI, ICU-acquired infection; MARS, Molecular Diagnosis and Risk Stratification of Sepsis; PBMC, peripheral blood mononuclear cell; scRNA-seq, single-cell RNA sequencing.

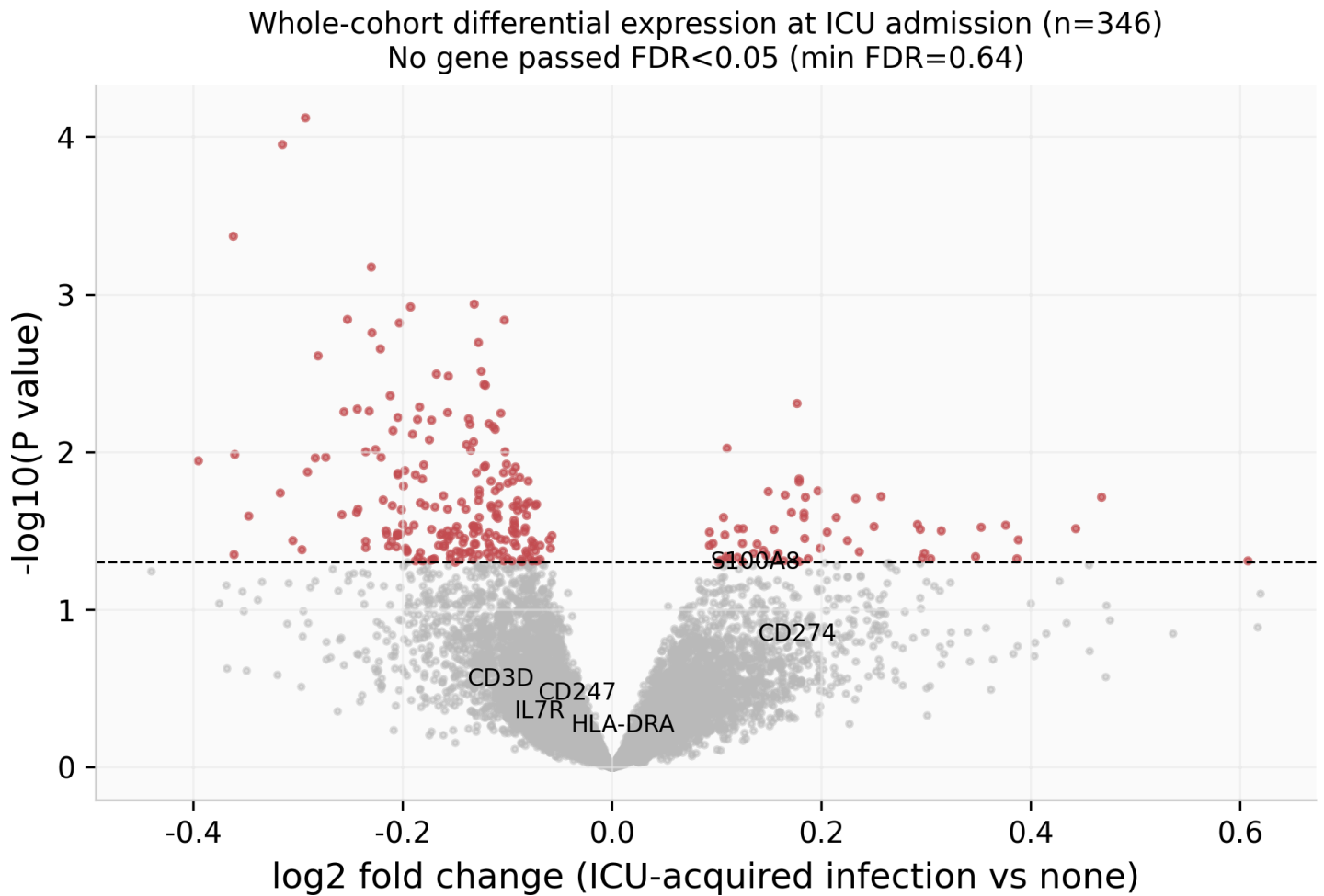


Figure 2

Whole-cohort differential expression at ICU admission. The volcano plot compares baseline whole-blood gene expression between 50 patients who subsequently developed ICU-AI and 296 patients who did not in [GEO: GSE65682]. Each point represents one gene. The x-axis shows the log2 fold change for ICU-AI versus no ICU-AI, and the y-axis shows the $-\log_{10}$ nominal P value. Red points indicate genes with nominal $P < 0.05$; the horizontal dashed line indicates $P = 0.05$. No gene remained statistically significant after Benjamini–Hochberg correction (minimum FDR=0.64). Selected immune-related genes are labeled. Abbreviations: FDR, false discovery rate; ICU-AI, ICU-acquired infection.

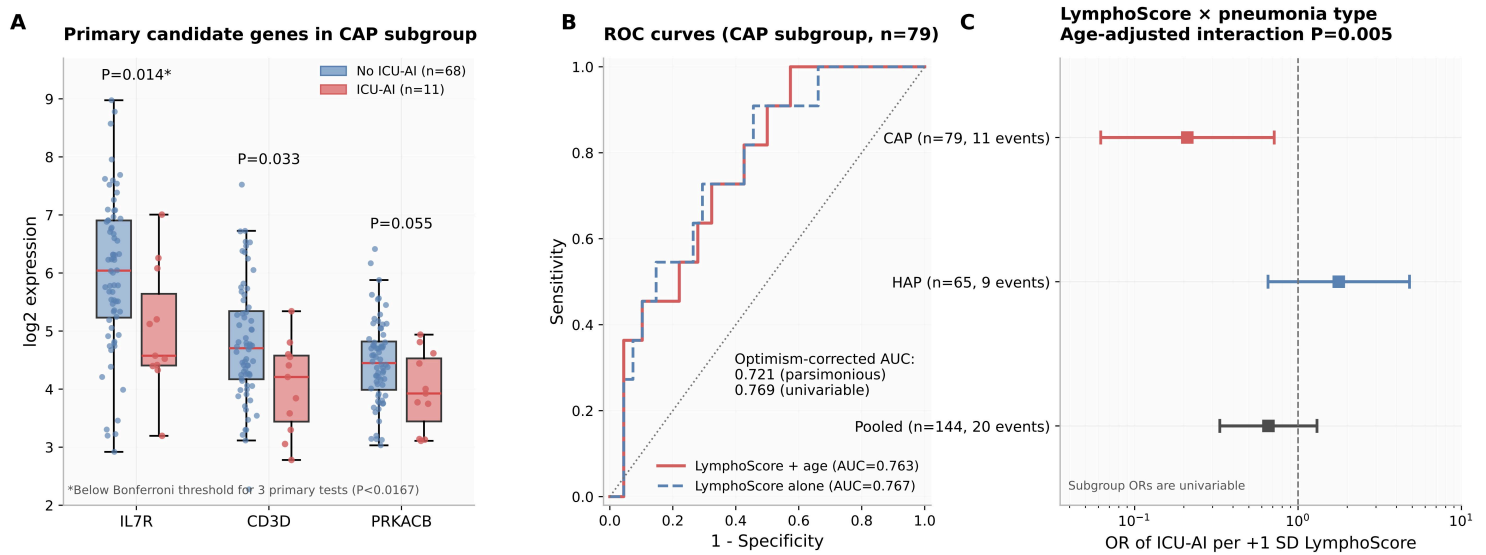


Figure 3

Candidate gene expression, model discrimination, and etiological interaction in the CAP subgroup. (A) Boxplots show log₂ expression of the three primary candidate genes (IL7R, CD3D, and PRKACB) in patients with CAP-induced sepsis according to subsequent ICU-AI status. Boxes represent medians and IQRs, whiskers extend to 1.5 times the IQR, and dots represent individual patients. P values were calculated using the Mann-Whitney U test. The Bonferroni-adjusted significance threshold for the three primary candidates was P<0.0167. (B) ROC curves show discrimination of the univariable model (LymphoScore alone) and the parsimonious model (LymphoScore plus age). Apparent AUCs are shown in the legend, and bootstrap optimism-corrected AUCs are annotated in the panel. (C) Forest plot shows ORs and 95% CIs for ICU-AI per 1-SD increase in LymphoScore in the CAP subgroup, HAP subgroup, and pooled pneumonia cohort. The x-axis is shown on a log scale, and the dashed vertical line indicates an OR of 1. Subgroup-specific ORs were estimated using univariable logistic regression, whereas the interaction P value was obtained from an age-adjusted logistic regression model using a likelihood-ratio test. Abbreviations: AUC, area under the receiver operating characteristic curve; CAP, community-acquired pneumonia; CI, confidence interval; HAP, hospital-acquired pneumonia; ICU-AI, ICU-acquired infection; IQR, interquartile range; OR, odds ratio; ROC, receiver operating characteristic; SD, standard deviation.

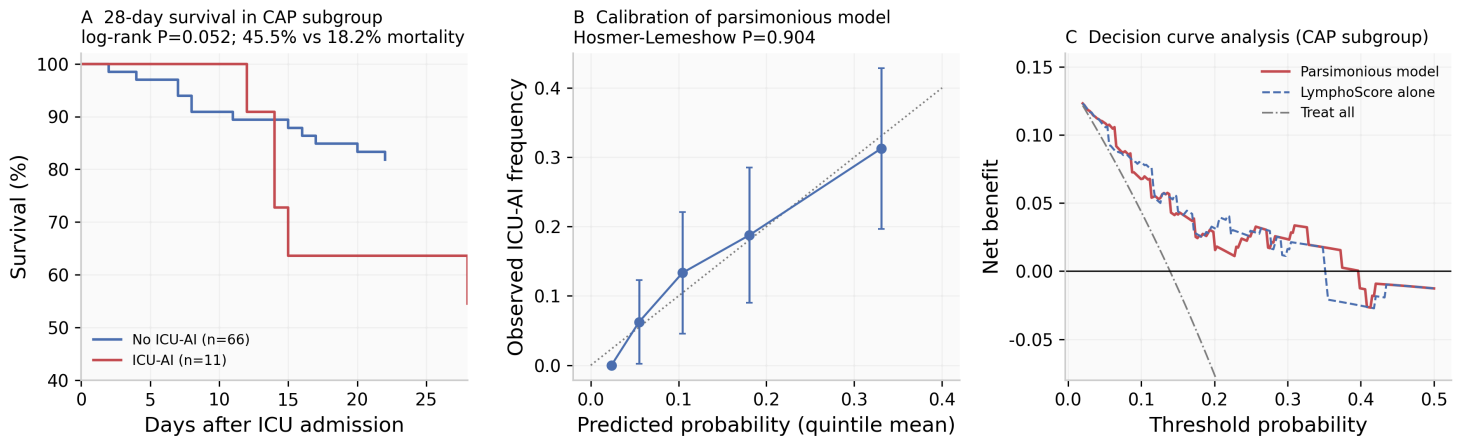


Figure 4

Survival, calibration, and decision curve analysis in the CAP subgroup. (A) Kaplan–Meier curves show 28-day survival according to ICU-AI status among 77 patients with available survival data. Mortality was 45.5% (5/11) in patients with ICU-AI and 18.2% (12/66) in those without ICU-AI; the P value was calculated using the log-rank test. (B) Calibration plot of the parsimonious model, which included LymphoScore and age. Points represent quintile mean predicted probabilities and corresponding observed ICU-AI frequencies; vertical bars indicate 95% CIs for the observed frequencies. The dotted line represents perfect calibration. Calibration was assessed using the Hosmer–Lemeshow test ($P=0.904$). (C) Decision curve analysis shows the net benefit of the parsimonious model and LymphoScore alone across threshold probabilities. The “treat all” strategy and the zero-net-benefit line are shown for reference. Abbreviations: CAP, community-acquired pneumonia; CI, confidence interval; ICU-AI, ICU-acquired infection.

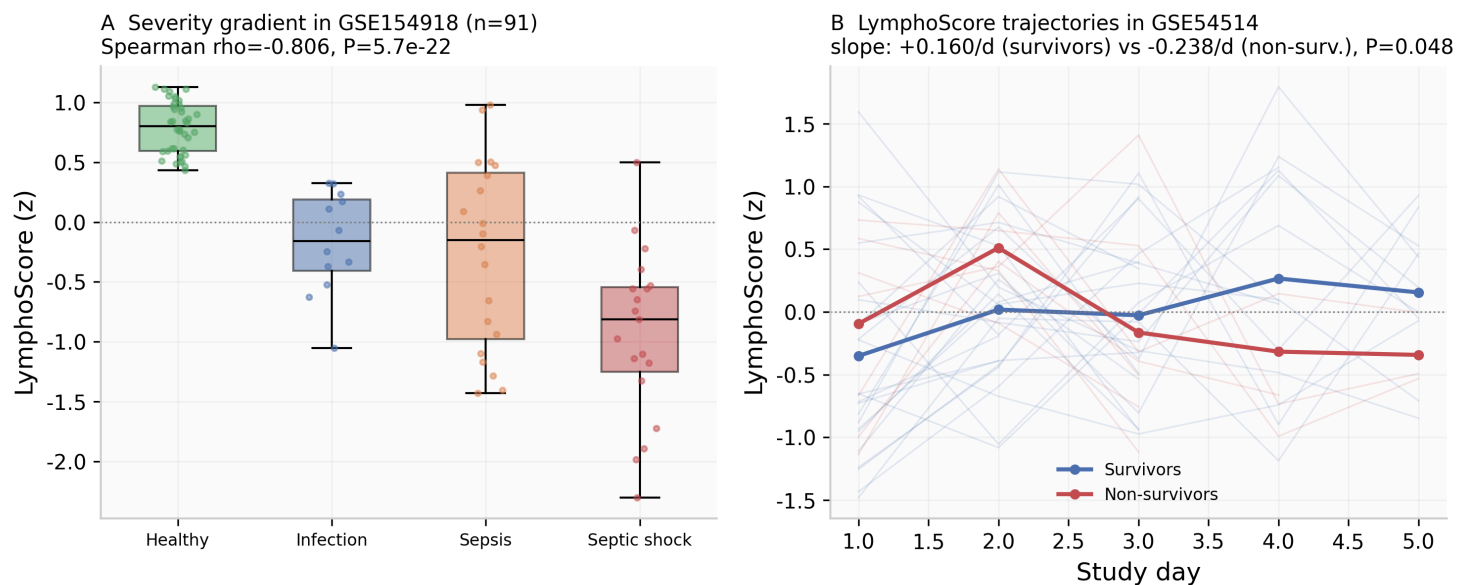


Figure 5

Endpoint-hierarchy external assessment of LymphoScore. (A) Boxplots show LymphoScore across the disease severity gradient in [GEO: GSE154918] (n=91): healthy controls, uncomplicated infection, sepsis, and septic shock. Boxes represent medians and IQRs, whiskers extend to 1.5 times the IQR, and dots represent individual samples. The monotonic association with disease severity was assessed using Spearman’s rank correlation ($\rho=-0.806$, $P=5.7\times10^{-22}$). (B) Longitudinal LymphoScore trajectories in [GEO: GSE54514] are shown for survivors and non-survivors. Gray lines represent individual patients, and colored lines represent group means. Slopes were estimated for patients with at least three longitudinal measurements; the mean slope was +0.160/day in survivors (n=22) and -0.238/day in non-survivors (n=7). Groups were compared using the Mann–Whitney U test ($P=0.048$). Abbreviations: IQR, interquartile range.

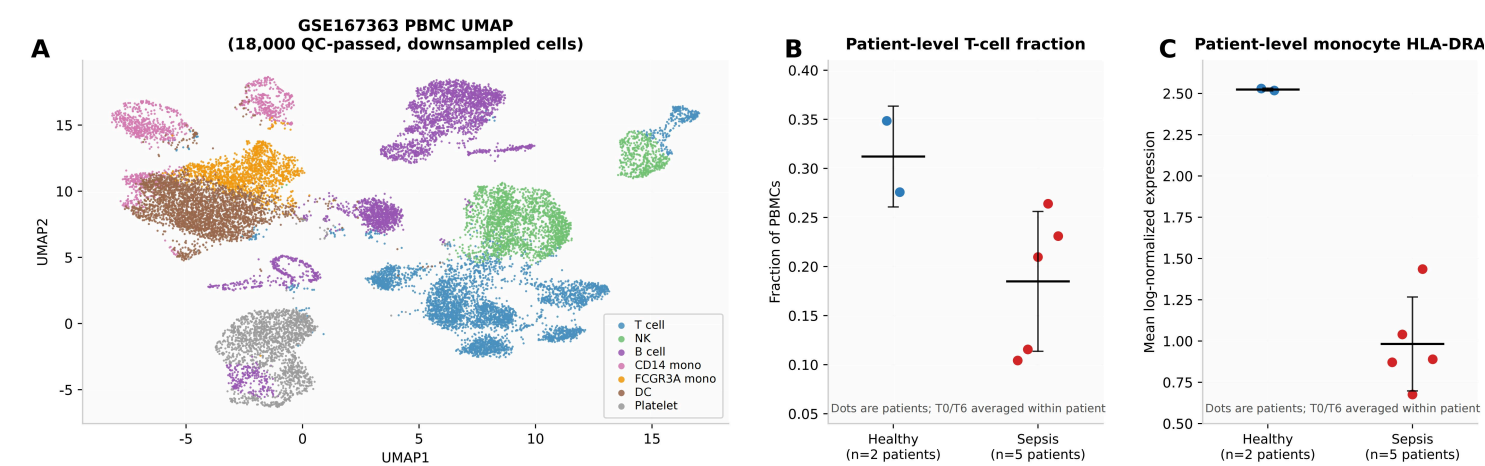


Figure 6

Exploratory patient-level single-cell anchoring of the sepsis immunosuppression phenotype in [GEO: GSE167363]. (A) UMAP visualization of 18,000 QC-passed, downsampled PBMCs, colored by annotated cell type. (B) Patient-level T-cell fraction in healthy controls (n=2 patients) and patients with sepsis (n=5 patients). (C) Patient-level mean log-normalized monocyte HLA-DRA expression in healthy controls and patients with sepsis. Cell-type proportions and marker expression were calculated from all QC-passed cells in each sample. For septic patients with T0 and T6 samples, values were averaged within each patient before plotting; dots represent individual patients, horizontal lines indicate group means, and error bars indicate SDs. Because only two healthy controls and five septic patients were available, no inferential P values are reported and these results should be interpreted as exploratory cellular anchoring. Abbreviations: CD, cluster of differentiation; DC, dendritic cell; FCGR3A, Fc fragment of IgG receptor IIIa; HLA-DRA, major histocompatibility complex, class II, DR alpha; mono, monocyte; NK, natural killer; PBMC, peripheral blood mononuclear cell; QC, quality control; SD, standard deviation; T0/T6, 0 and 6 h after sepsis recognition; UMAP, uniform manifold approximation and projection.

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