Dynamic changes in human single-cell transcriptional signatures during fatal sepsis

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Abstract

Systemic infections, especially in patients with chronic diseases, may result in sepsis: an explosive, uncoordinated immune response that can lead to multisystem organ failure with a high mortality rate. Patients with similar clinical phenotypes or sepsis biomarker expression upon diagnosis may have different outcomes, suggesting that the dynamics of sepsis is critical in disease progression. A within-subject study of patients with Gram-negative bacterial sepsis with surviving and fatal outcomes was designed and single-cell transcriptomic analyses of peripheral blood mononuclear cells (PBMC) collected during the critical period between sepsis diagnosis and 6 h were performed. The single-cell observations in the study are consistent with trends from public datasets but also identify dynamic effects in individual cell subsets that change within hours. It is shown that platelet and erythroid precursor responses are drivers of fatal sepsis, with transcriptional signatures that are shared with severe COVID-19 disease. It is also shown that hypoxic stress is a driving factor in immune and metabolic dysfunction of monocytes and erythroid precursors. Last, the data support CD52 as a prognostic biomarker and therapeutic target for sepsis as its expression dynamically increases in lymphocytes and correlates with improved sepsis outcomes. In conclusion, this study describes the first single-cell study that analyzed short-term temporal changes in the immune cell populations and their characteristics in surviving or fatal sepsis. Tracking temporal expression changes in specific cell types could lead to more accurate predictions of sepsis outcomes and identify molecular biomarkers and pathways that could be therapeutically controlled to improve the sepsis trajectory toward better outcomes.

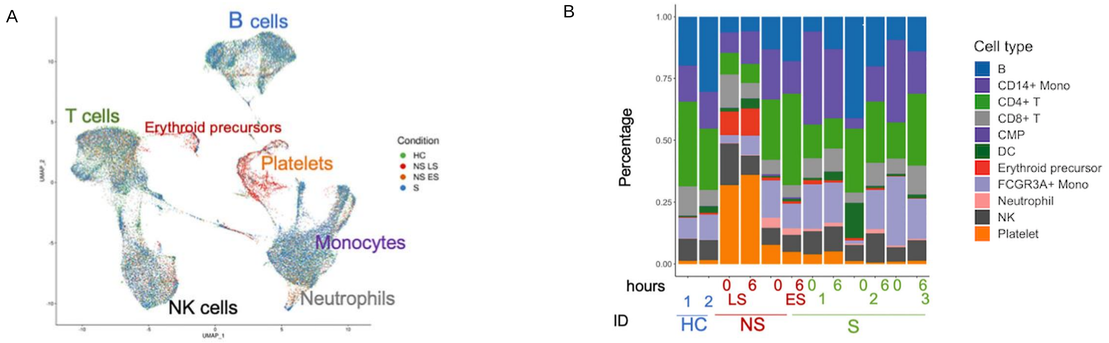
Introduction

	Non-sepsis control (n=2)		Sepsis non-survivor (n=2)		Sepsis survivor (n=3)		
Gender	Male	Female	Male	Female	Male	Female	Female
Age range	35-40	45-50	90-95	65-70	45-50	65-70	70-75
Sepsis etiology	n/a	n/a	E.coli bacteremia		E.coli bacteremia		
APACHE II	n/a	n/a	18	38	31	41	19
SOFA	n/a	n/a	11	16	11	15	7
Time of death (days post enrollment)	n/a	n/a	<30	1	n/a	n/a	n/a
Plasma cytokines (ng/mL)							
Resistin	22.5	36.7	202	135.9	147	281	92
IL-6	N.D	0.002	30.2	142.3	133	2.48	0.31
IL-8	0.03	0.026	6.65	27.2	41.7	0.61	0.4
IL-10	N.D.	N.D.	0.13	9.71	0.52	0.15	0.39
LPS-induced TNFα (ng/mL)							
TNFa	0.656	0.979	0.45	0.0046	n/a	0.047	0.1

Table 1. Characteristics of enrolled sepsis patients in the study.

Results

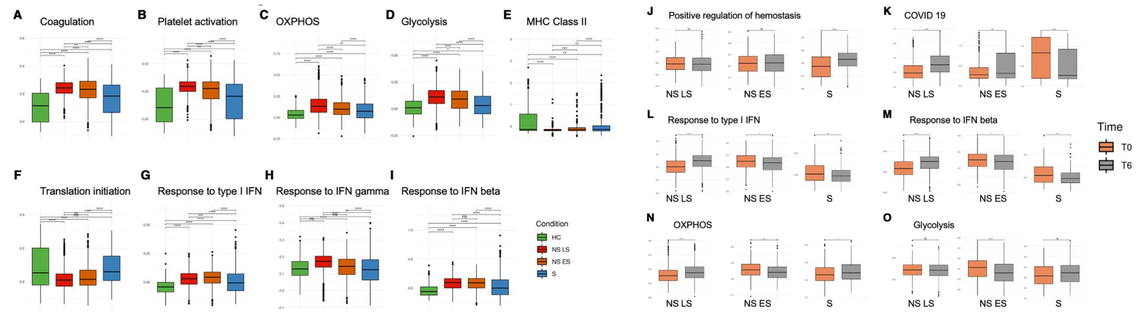
Figure 1. Single-cell transcriptional profiling of PBMC from healthy controls and gram-negative sepsis patients.



(A) Sample of origin UMAP representation of all merged samples. Cells were colored by the condition.

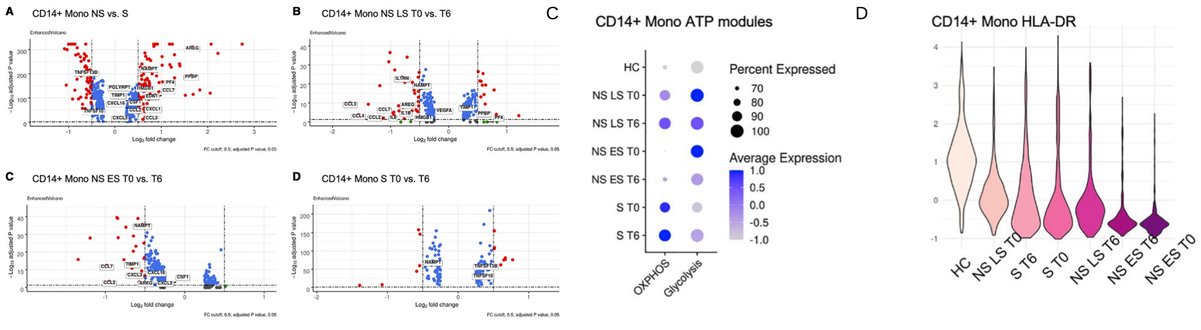
(B) Bar plots showing the fraction of each sample.

Figure 2. Platelet transcriptional changes over 6 h are associated with sepsis severity.



(A– I) Comparisons of pathway module scores across four conditions in platelets. (J–O) Pathway module scores comparison between T0 vs. T6 in platelets. The differences in scores associated with adjusted P-values below 0.05, 0.01, 0.001, and 0.0001 are indicated as *, **, ***, and ****, respectively and "ns" – not significant.

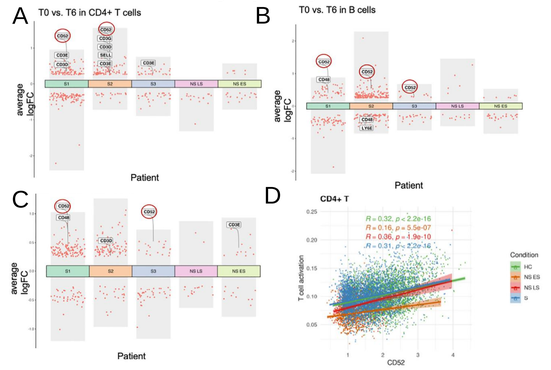
Figure 3. Fatal sepsis patients exhibit immunosuppressive pathways in monocytes.



(A-D) Differential expression genes in CD14+ monocytes from (A) NS versus S, (B) NS LS T0 versus T6, (C) NS ES T0 versus T6, (D) S T0 versus T6. (C) The percentage of cells with ATP-related pathway modules in CD14+ monocytes across healthy controls and sepsis conditions at T0 and T6. (D) HLA-DR-related genes expression in CD14+ monocytes across healthy controls and sepsis conditions at T0 and T6.

Results

Figure 4. CD52 is a prognostic biomarker for beneficial outcomes in sepsis and is associated with lymphocyte activation.



(A-C) Differential gene expression analysis showing up- and down-regulated genes with |log2FC| > 0.25 and adjusted P-value < 0.05 across all 5 sepsis patients between T0 and T6 in CD4+ T cells, B cells, and CD8+ T cells. (D) CD52 expression and its correlation with the T cell activation pathway module score in CD4+ T cells across four conditions.

Conclusions

- The immune subsetting data by scRNA-seq indicated that lymphocyte subsets were reduced in sepsis, especially in fatal outcomes, and identified the emergence of platelet and erythroid precursors in late-stage fatal sepsis.(Fig. 1).
- Confirmed the theory that platelet coagulation, activation, and energy consumption are functionally linked to sepsis disease severity and identifies shared pathways with COVID-19 dis- ease progression. Further, our timed analysis reveals that these platelet responses are dynamic, changing within a 6-h window, especially in the late stages of fatal sepsis. (Fig. 2).
- Revealed dynamic transcriptional changes in monocytes within 6 h of sepsis diagnosis, which follow opposite trends in surviving and fatal outcomes. Fatal sepsis is associated with heightened inflammatory and metabolic activity that is down- regulated over time, while improved sepsis out- comes are associated with the restoration of monocyte function within 6h.(Fig. 3).
- Our data indicated that increased CD52 expression within hours of sepsis recognition is associated with improved sepsis outcomes. Here, CD52 may act to promote restoration of protective lymphocyte responses, and therefore may serve both as a biomarker for sepsis progression, or as a therapeutic target to promote immune homeostasis.(Fig. 4).

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