DISCOVERY OF PERTURBED SIGNALING STATES IN BLOOD IMMUNE CELLS FROM CRITICALLY ILL PATIENTS THAT DEVELOP SEPSIS AND ARDS

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Background and Introduction

- Critically ill, immune compromised patients are at high risk of developing opportunistic bacterial infections, sepsis, acute respiratory distress syndrome (ARDS), and other life-threatening complications.
- To gain new insights into perturbed immune cell phenotypes associated with the development of sepsis, ARDS, and mortality in patients, we applied a whole blood mass cytometry (CyTOF) approach to comprehensively measure signaling states in all peripheral immune cell types at single cell resolutions.
- We introduce this **Functional CyTOF** method by profiling whole blood samples from non-sepsis and sepsis ICU patients for perturbed phosphosignaling patterns in specific immune cell subsets at baseline or after LPS or PMA/Ionomycin stimulation
- *We hypothesize that this approach will provide new insights into the "immune health" of immune compromised patients that develop sepsis or complications of sepsis in the medical ICU to help better understand the functional immune changes contributing to sepsis, ARDS, and mortality.*

Methods

- Blood samples were prepared from patients in the medical ICU at Brigham and Women's Hospital with the indicated demographics and clinical metadata
- 0.2 mL of whole blood was incubated with 5µg/mL of *E. coli* lipopolysaccharide (LPS, O26:B6), PMA/Ionomycin (10ng/mL, 1 µM) or no stimuli for 10 minutes
- Proteomic stabilizer buffer (Smart Tube, Inc) was added to stop signaling and stabilize cells for freezing at -80°C
- Samples were thawed and prepared for CyTOF staining with a 48-marker antibody panel (10 phospho-protein markers and 38 immune cell phenotyping markers)
- CyTOF staining data was analyzed using our OMIQ analysis platform for computational clustering, dimensional reduction, and statistics
- Plasma samples were analyzed by a 41 cytokine Luminex panel

Results

- We applied a **Functional CyTOF** workflow to detect phosphosignaling profiles in all blood immune cells at single-cell resolutions at baseline
- Immune cell subset abundances were not different between non-sepsis and sepsis patients, but non-survivors had significantly higher circulating neutrophils and lower monocyte percentages
- The **p38MAPK, p44/42MAPK** signaling pathways were identified as being perturbed in specific immune cell types in sepsis patients (P-p38MAPK high in monocytes, P-p44/42MAPK low in neutrophils)
- Neutrophils were the primary immune cell type showing perturbed p38MAPK and p44/42MAPK signaling in ARDS or non-survivors - high P-p38MAPK in ARDS and non-survivors and low P-p44/42MAPK in ARDS patients
- Multiple cytokine profiling assays identified different cytokine levels between non-sepsis and sepsis patients, alive and non-survivors, and non-ARDS and ARDS patients. Cytokines most significantly affected included: **IL-18, MIP3α, Rantes, IP-10, Gro-α, Flt3L, PDGF-AA, and PDGF-BB.**

Conclusions and Future Directions

1. We demonstrate that **Functional CyTOF** approaches to study altered signaling (shown here), cytokine expression, or epigenetic marks in small volumes of clinical samples has potential to reveal new insights into the immune health of individuals with disease
2. Future work done in collaboration with the BWH medical and surgical ICU will apply functional CyTOF and other systems immunology approaches to advance our understanding immune dysfunction in trauma, sepsis, and other immune compromised patients

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Functional mass cytometry (CyTOF) shows perturbed phosphosignaling phenotypes in neutrophils and monocytes in whole blood from sepsis patients

Brigham and Women's Hospital
Harvard Medical School
https://ledererlab.bwh.harvard.edu/

Patient Demographics and Clinical Metadata

Gender	Average Age	% Cancer+	Non-Sepsis ICU Controls	Sepsis	Sepsis + ARDS	APACHE	Non-Survival
12 Male (46.9%)	59.1	87%	25%	75%	67%	23.8	41.70%
15 Female (53.1%)	58.2	20%	33%	66.70%	10.00%	23.4	26.70%

Functional Mass Cytometry (CyTOF) Workflow

Phospho-Signaling Functional CyTOF Panel

Marker	Cellular Location	Metal
CD45	Cell surface	89Y
CD172ab	Cell surface	111Cd
CD4	Cell surface	112Cd
CD20	Cell surface	113Cd
CD8	Cell surface	114Cd
CD3	Cell surface	115In
CD56	Cell surface	116Cd
CD66b	Cell surface	141Pr
Ki-67	Cytoplasm	142Nd
CD134	Cell surface	143Nd
CD39	Cell surface	144Nd
CD64	Cell surface	145Nd
CD14	Cell surface	146Nd
CD10	Cell surface	147Sm
p-NF-kB P65 (S536)	Cytoplasm	148Nd
CD18	Cell surface	149Sm
CD11c	Cell surface	150Nd
p-LCK	Cytoplasm	151Eu
HLA-A,B,C	Cell surface	152Sm
p-Jak2	Cytoplasm	153Eu
CD15	Cell surface	154Sm
CD35	Cell surface	155Gd
Cleaved Caspase-3	Cytoplasm	156Gd
CD16	Cell surface	157Gd
IL-17R	Cell surface	158Gd
CD62L	Cell surface	159Tb
p-Stat1 (Y701)	Cytoplasm	160Gd
CD63	Cell surface	161Dy
CD123	Cell surface	162Dy
CD121b	Cell surface	163Dy
CD182	Cell surface	164Dy
CD177	Cell surface	165Ho
p-p38MAPK (T180/Y182)	Cytoplasm	166Er
p-p44/42MAPK (T202/Y204)	Cytoplasm	167Er
S100A8/9	Cytoplasm	168Er
p-Stat3 (Y705)	Cytoplasm	169Tm
p-AKT (S473)	Cytoplasm	170Er
CD184	Cell surface	171Yb
CD274	Cell surface	172Yb
CD114	Cell surface	173Yb
p-Stat5a/b (Y964)	Cytoplasm	174Yb
p-S6 Ribo (S235/236)	Cytoplasm	175Lu
p-CREB (S133)	Cytoplasm	176Yb
FOXP3	Nucleus	194Pt
HLA-DR	Cell surface	195Pt
CD141	Cell surface	196Pt
CD34	Cell surface	198Pt
CD11b	Cell surface	209Bi

Workflow and Data Analysis

Phospho-CyTOF Workflow

0.2mL Blood
- Unstimulated
- LPS (5 µg/ml)
- PMA/Iono (10 ng/1 µM)

↓ 10 min. @37 C

Add Proteome Stabilization Buffer

↓ 10 min. @RT, -80C freezer

CyTOF Stain
- Thaw samples and lyse RBCs
- Batch stain samples with 48-marker CyTOF antibody panel with sample barcoding
- Collect single-cell staining data using CyTOF instrument (Standard BioTools)

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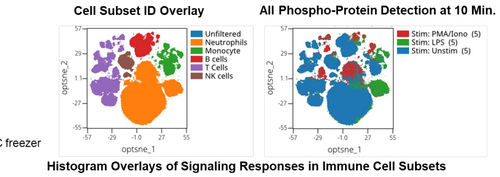
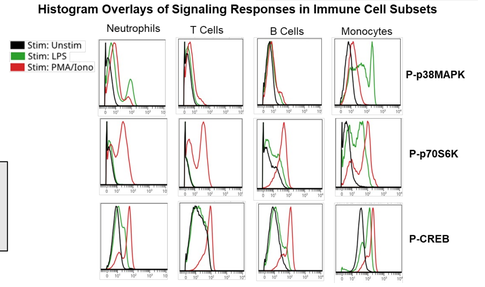
Data Analysis - OMIQ
- Data cleanup
- Clustering
- Cell Subset Identification by Marker Profiles
- Dimensional Reduction
- Data Visualization and Statistics

Optimization Study With Blood From Normal Healthy Volunteers





FIGURES AND TABLES

Figure 1: Whole Blood Immune Cell Subset Profiles Detected by CyTOF

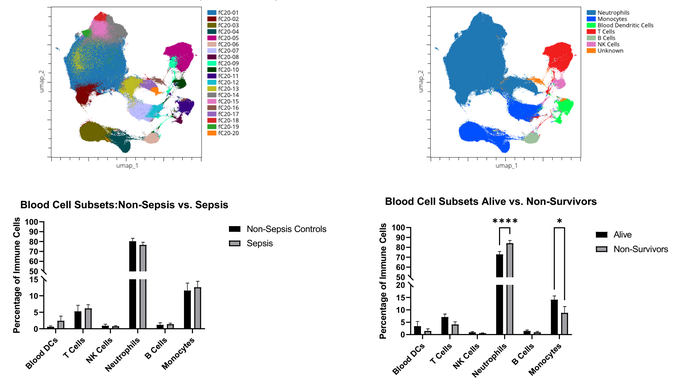


Figure 2: Phosphosignaling staining profiles in baseline (unstimulated), LPS, or PMA/Iono stimulated whole blood samples from all patients

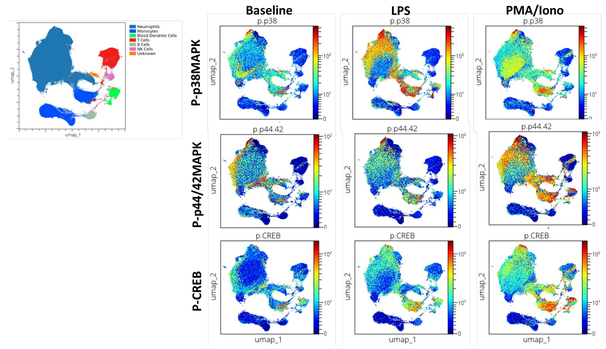


Figure 3: Baseline signaling profiles in blood immune cells from non-sepsis vs. sepsis patients

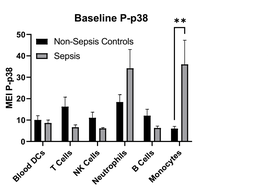


Figure 4: Differences in baseline blood immune cell signaling phenotypes between patient subsets (ARDS and non-survivors)

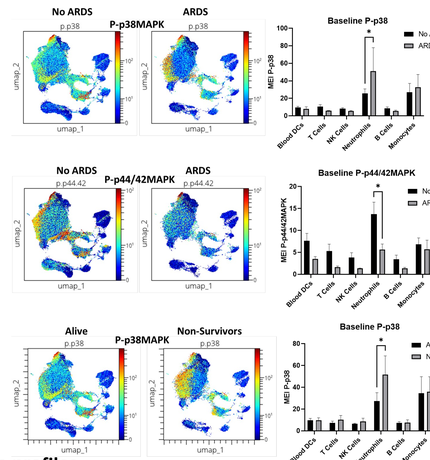


Figure 5: Patient plasma cytokine profiles