Epigenetic assays identify leukocyte subpopulations that differentiate outcomes in a West African sepsis cohort

J. Chenoweth[1], J. Brandsma[1], S. Krishnan[1], J. Michalska[2], U. Hoffmueller[2], J.Achenbach[2], A. Rosselló Chornet[2], G. Oduro[3], N. Adams[4], D.Clark[1]

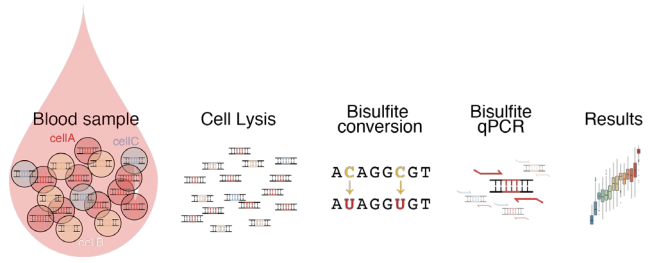
[1] Austere environments Consortium for Enhanced Sepsis Outcomes (ACESO), The Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc., Bethesda, MD, USA
[2] Precision for Medicine GmbH, Berlin, Germany. [3] Accident and Emergency Department, Komfo Anokye Teaching Hospital, Kumasi, Ghana. [4] Directorate for Department of Defense Infectious Disease Research, Naval Medical Research Center, Silver Spring, MD.

INTRODUCTION

Although understanding of the sepsis host immune response has advanced considerably, it has not translated into effective sepsis care and management. A barrier to progress is the broad definition of the sepsis syndrome, which encompasses an array of features. Cellular immunophenotyping has emerged as a powerful tool for sepsis endotyping and overcoming the problem of heterogeneity. However, technologies such as single-cell RNA-sequencing and cytometry by time-of-flight are not readily accessible in low resources settings. More recently developed epigenetic quantitative polymerase chain reaction methods enable immune cell characterization and counting by evaluating cell type-specific unmethylated DNA[1]. This approach is suited for low-resource settings and low- and middle-income countries. To enable this tool, we sought to conduct comprehensive epigenetic cellular immunophenotyping to assess innate and adaptive immune cells in sepsis patients enrolled in an observational sepsis study in a tertiary care hospital in Ghana.

METHODS

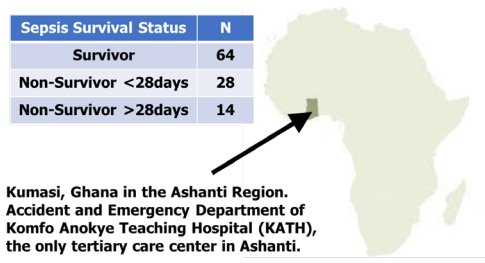
We conducted comprehensive epigenetic cellular immunophenotyping of innate and adaptive immune cells in sepsis patients enrolled in an observational study of sepsis in Ghana implemented by the Austere environments Consortium for Enhanced Sepsis Outcomes (ACESO)[2]. Fourteen epigenetic assays were used to analyze whole blood of 103 subjects upon admission to the emergency department with suspected infection and two or more SIRS criteria[3]. Up to five serial samples (0, 1, 3 and 28 days, 6 and 12 months) were analyzed from whole blood or dried blood on filter paper using published protocols[1].

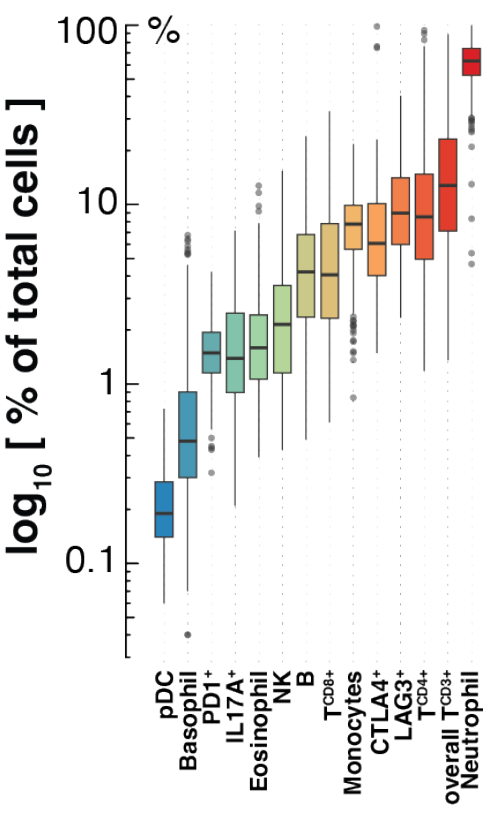


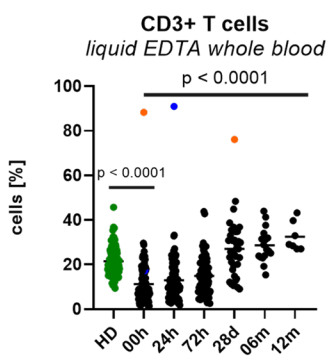
RESULTS

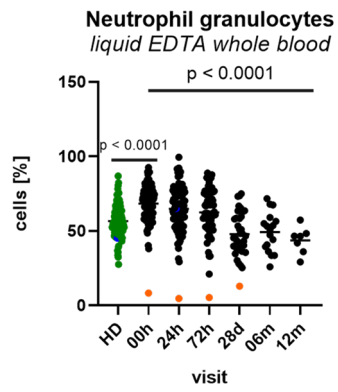
Fig 1. Epigenetic Immunophenotyping

Sepsis Survival Status	N
Survivor	64
Non-Survivor <28days	28
Non-Survivor >28days	14





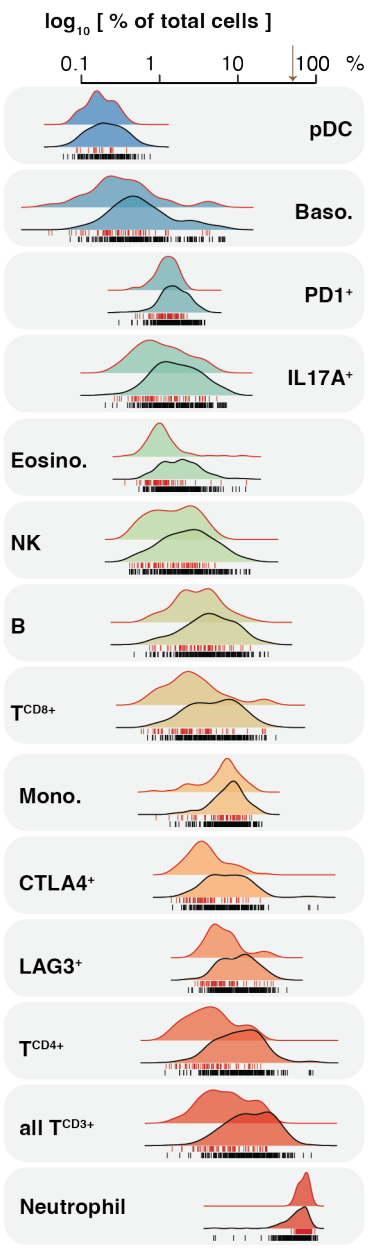


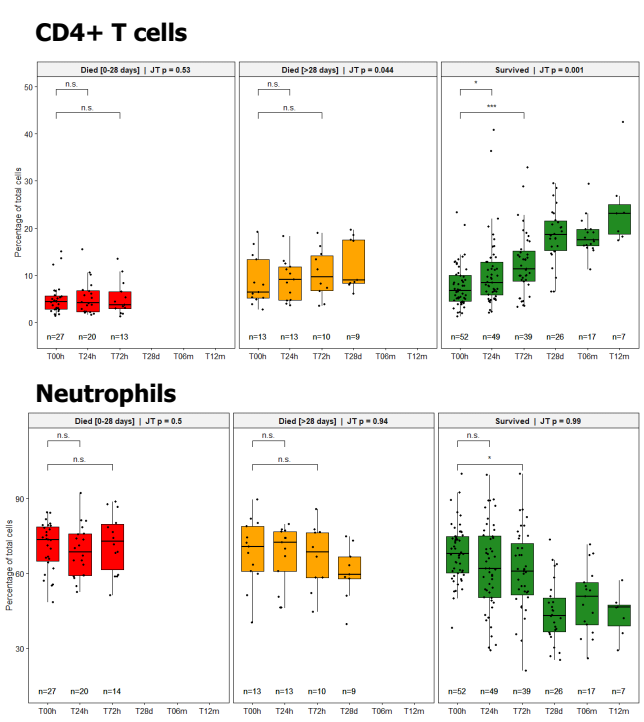


Epigenetic Assay	Sepsis vs. Healthy Donors	Direction vs. Healthy
T-Cells CD3+	***	Down
T-helper CD4+	***	Down
Cytotoxic T CD8+	***	Down
B cells	***	Down
NK cells	***	Down
Th17	***	Down
Neutrophils	***	Up
Basophils	n.s	n.s
Eosinophils	n.s	n.s
Monocytes	n.s	n.s
PD1+ cells	**	Down
CTLA4+ cells	n.s	n.s
LAG3+ cells	**	Down
pDendritic Cells	n.s	n.s

Fig 1. Epigenetic qPCR for 14 cell types was performed on 314 whole blood samples from 103 subjects in a Ghana sepsis cohort generating 4396 data points. Cell types including total CD3+ T-Cells and neutrophil granulocytes were significantly different between healthy donors (HD) at enrollment. *=<.0001, **<.005 T-Test**

Fig 3. Serial Sampling through Time



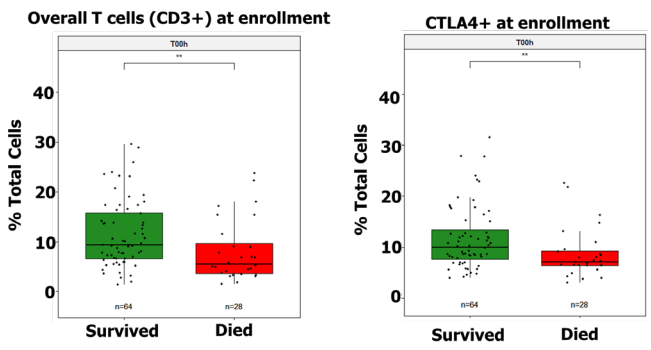


Trends Over First 72h by Outcome

Epigenetic Assay	Non-Survivor <28d	Non-Survivor >28d	Survivor
T-Cells CD3+	n.s.	n.s.	**
T-helper CD4+	n.s.	*	**
Cytotoxic T CD8+	n.s.	n.s.	*
B cells	n.s.	n.s.	*
NK cells	n.s.	n.s.	n.s.
Th17	n.s.	n.s.	*
Neutrophils	n.s.	n.s.	n.s.
Basophils	n.s.	n.s.	*
Eosinophils	n.s.	*	**
Monocytes	n.s.	n.s.	n.s
PD1+ cells	n.s.	n.s.	**
CTLA4+ cells	n.s.	n.s.	**
LAG3+ cells	n.s.	n.s.	n.s.

Fig 3. Density plot (left) showing changes in cell percentages in survivors and non-survivors (<28d). Boxplots of cell percentages in survivors and non-survivors (<28d and >28d). *=<.05, **<.001, *<.0001 KW Test. JT= Jonckheere-Terpstra test for trends over first 72h.**

Fig 2. Immunophenotypes by 28day Mortality



Epigenetic Assay	Survivor vs. 28d Non-Survivor
T-Cells CD3+	**
T-helper CD4+	**
Cytotoxic T CD8+	**
B cells	n.s
NK cells	n.s
Th17	n.s
Neutrophils	n.s
Basophils	n.s
Eosinophils	**
Monocytes	n.s
PD1+ cells	n.s
CTLA4+ cells	**
LAG3+ cells	*

Fig 2. Cell types including total CD3+ T-Cells and CTLA4+ cells were significantly different between survivors and non-survivors (<28d) at enrollment. **<.005 KW Test

Fig 4. Prediction

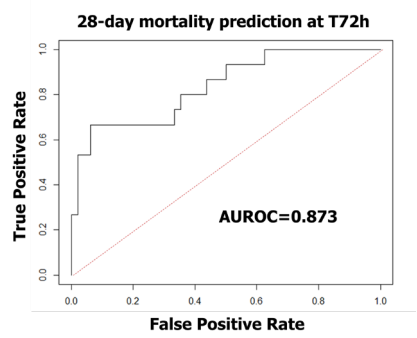
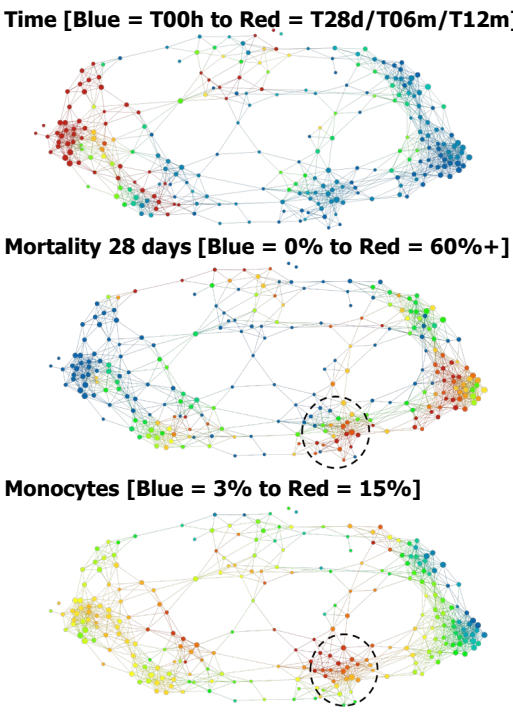


Fig 4 (top). Binary logistic regression model using T-helper cell (CD4+), Cytotoxic T cell (CD8+), PD1+ cell and CTLA4+ cell data.

Fig 5 (right). Topological Data Analysis (TDA) decomposition of all samples shows cell-type differences in non-survivor subgroups.

Fig 5. Decomposition



CONCLUSIONS and FUTURE

Our results show that epigenetic immune cell profiling is a promising new tool for diagnostic and prognostic profiling of sepsis subjects in low resource settings. Neutrophilia and lymphopenia observed in sepsis subjects is observed in the Ghanaian cohort using this novel approach, In addition, results between frozen whole blood and dried blood on filter paper are highly comparable (data not shown) further suggesting that this approach is suitable for low resource settings. Prognostic models using these data could be improved through subject stratification using tools such as TDA and combination with host gene expression and proteomics. Future analysis will use these data to identify clinically relevant endotypes.

REFERENCES

[1]Baron et al. Epigenetic immune cell counting in human blood samples for immunodiagnostics. Sci. Transl. Med., 1 Aug 2018, 10 eaan3508.
[2]Krishnan et al. Austere environments Consortium for Enhanced Sepsis Outcomes. Shock. Mar;53(3):377-378.
[3]Blair et al. Screening tools for predicting mortality of adults with suspected sepsis: an international sepsis cohort validation study. BMJ Open. 2023;13:e067840. doi:10.1136/bmjopen-2022-067840.

 This project was funded by the Defense Health Agency's Joint Program Committee-2/Military Infectious Diseases Research Program (JPC-2/MIDRP) and CDMRP award PR191965 under the HJF- (DoD - Navy) Cooperative Agreement N626451920001. The study protocol NMRC.2016.004-GHA were approved by the NMRC Institutional Review Board.