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Genomics, Microbiomics, and Bioenergetics-Based Personalized Treatment for Head Trauma Patients at Risk for Sepsis

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14. ABSTRACT Systemic, non-neurological complications such as sepsis and organ failure are common after severe traumatic brain injury (TBI). Sepsis occurs in 50-75% of patients with severe TBI and can lead to multiple organ system failure, but the search for early-detection biomarkers for sepsis is ongoing. This clinical study examines the correlations between blood cell gene expression and bioenergetics with the clinical observations and long-term outcomes of severe TBI patients admitted to the R. Adams Cowley Shock Trauma Center in order to identify prognostic biomarkers for sepsis and organ failure. Our hypothesis states that gene expression and mitochondrial health of circulating blood cells sense stress in patients and may serve as biomarkers of human pathologies such as sepsis. Gene expression is investigated by whole blood ribonucleic acid (RNA) isolation and analysis via Nanostring nCounter® over the course of 7 days post-injury. Similarly, fresh blood is drawn over the first 7 days for isolation and bioenergetic examination of peripheral blood mononuclear cells (PBMCs). Through the use of machine learning techniques, these data sets were compared to clinical data with the purpose of identifying novel biomarkers as early diagnostic tools and improving outcomes of patients with severe TBI. The results of these studies are detailed in the following technical report.

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1.0 EXECUTIVE SUMMARY

Background: Severe traumatic brain injury (TBI) is often followed by sepsis and/or organ failure (SOF) which occurs in over half of these patients and frequently leads to death. Little is known about the factors that control this transition. One possibility is that patterns of gene expression systemically regulate energy metabolism, immune responses, and inflammation that either repair or further damage critical organs. Other factors, such as the metabolic state of circulating blood cells, may also provide insight into the physiological responses to SOF. Currently available technologies, such as the Agilent Seahorse XFe24 Extracellular Flux Analyzer and Nanostring gene expression assays, allow the monitoring of these metrics on an individual level. We posited actionable links between genomic and metabolic findings and physiologic response to develop patient-specific approaches to triage, therapeutics, and supportive care of Wounded Warriors so that potentially survivable mortality could be reduced, and long-term outcomes would be improved.

Methods: We enrolled 45 adult patients at the University of Maryland Shock Trauma Center who were at high risk for sepsis due to the initial diagnosis of severe TBI. Patients were monitored for up to 30 days, during which time an array of clinical metrics were recorded including diagnosis of SOF. Ultimately, 17 of the enrolled patients developed SOF while under observation. Blood samples were obtained over 7 days after admission for blood cell bioenergetics and transcriptomic analyses. For bioenergetics assays, fresh blood samples were divided into platelets and peripheral blood mononuclear cells (PBMCs). These cells were analyzed for oxygen consumption rate (OCR; aerobic metabolism) and extracellular acidification rate (ECAR; anaerobic metabolism) using the Seahorse Analyzer. For transcriptomics assays, blood was drawn into Tempus™ ribonucleic acid (RNA) stabilization tubes. The isolated RNA was analyzed utilizing Nanostring technology and a custom 800-gene transcript panel that included genes involved in inflammation, immunity, mitochondrial function, and other potentially associated pathways. We then applied a machine learning approach in which clinical outcomes were compared to bioenergetics metrics and gene expression profiles to identify prognostic biomarkers of physiological responses to the diagnosis of SOF. Finally, several samples from our enrolled patient population were banked and are available for future analyses.

Results: We conducted preliminary analyses of key demographic and Day 0 clinical variables, including vital signs and laboratory values, using a series of independent samples t-tests or chi square tests. Of the demographic variables 100 percent (%) of the participants who were diagnosed with the combined SOF group were white. The Glasgow Coma Scale (GCS) scores on admission were lower in the SOF group. There were no differences in gender. Temperature was significantly lower in the SOF group and systolic blood pressure (SBP) was significantly higher. Blood calcium was significantly lower in the SOF group, and partial thromboplastin time (PTT) was significantly higher in the SOF group. There were no differences in glucose, white blood cell counts (WBC), red blood cell counts (RBC), hemoglobin, hematocrit, platelets, mean corpuscular volume (MCV), lymphocytes, monocytes, eosinophils, basophils, neutrophils, sodium chemistry, potassium chemistry, carbon dioxide, chloride chemistry, creatinine, lactate, and other variables.

We conducted a series of independent analyses of cellular bioenergetics data between patients that were never diagnosed with SOF (NonSOF) and those that were within 30 days. SOF

patients in general showed increased PBMC ECAR measurements soon after injury which persisted throughout the sampling period. Platelets did not show a lasting correlation between SOF diagnosis and any bioenergetic metric, although the trends over time for both aerobic and anaerobic respiration metrics were increased in the platelets of SOF patients compared to the NonSOF group.

Using platelet poor plasma derived from the bioenergetics cell isolation procedure, we measured the levels of different cytokines and evaluated association with SOF diagnosis. We found several cytokines that correlated to SOF diagnosis, although interleukin-6 and interleukin-16 were found to be higher soon after injury and were maintained at a significantly higher level throughout sampling.

Our blood transcriptomics analyses revealed several genes that were differentially expressed in different patient groups and had significantly different expression profiles over time. Of note, transcripts for genes GALNT14, PCOLCE2, and VNN1 showed positive trends in SOF patients while showing negative trends in NonSOF patients.

Finally, we compiled clinical, bioenergetic, and transcriptomic datasets to model the prognostic value of variables regarding SOF. Our models specified certain variables of all 3 categories that were predictive from an early time point for eventual SOF diagnosis. These models can be further refined with additional assays or analyses to bolster their predictive performance.

Conclusions and Future Directions: Although this study was affected by the overlapping COVID-19 pandemic which hindered enrollment, we were successful at identifying clinical and physiological variables that were associated with SOF with only 45 patients. Some of the significant indicators, such as PTT or cytokine levels, may be predictive of susceptibility to infection soon after injury, whereas the expression trends of genes such as GALNT14 could signal a descent into SOF. Finally, we have constructed a dataset of clinical metrics and a bank of biological samples from our patient population that can be further analyzed to identify physiological responses to SOF and potentially refine early critical care.

2.0 INTRODUCTION

Traumatic brain injury (TBI) is a leading cause of death and disability in the United States contributing to about 30% of all injury deaths. Each year, TBI afflicts at least 1.4 million individuals and accounts for 50,000 deaths and 235,000 hospitalizations. Members of the United States military are especially at risk for TBI with 352,612 TBI diagnoses among members in the United States (US) military between 2000 and 2016 [1]. Severely injured TBI patients, who appear otherwise equivalent upon admission, will classically dichotomize into those who survive without life-threatening complications and those who develop life-threatening complications, such as sepsis and/or organ dysfunction for unclear reasons [2]. These systemic complications are independent contributors to morbidity and mortality [3, 4]. Therefore, the prevention and treatment of these complications represents an important and potentially modifiable risk after TBI [4].

Systemic, non-neurological complications such as sepsis and related organ failure are common after severe TBI. Sepsis is "life-threatening organ dysfunction caused by a dysregulated host response to infection" [5], and occurs in 50-75% of patients with severe TBI [6-8]. TBI activates an inflammatory response, the intensity of which is related to the degree of the primary injury and subsequent secondary insults [9]. The exceptionally complex cascade of inflammatory events following TBI is mediated by the production and activation of a variety of processes including catecholamines, cytokines, adhesion molecules, and other multifunctional peptides. TBI causes both local and systemic release of inflammatory mediators [10, 11]. It is likely that the inflammatory response after TBI is also an important mediator of secondary systemic injury. The overall effect of this inflammatory response is depression of the immune system and suppression of immunity [6, 12].

Organ dysfunction or failure can occur without infection. In a prospective study of patients with severe TBI, the majority manifested dysfunction of at least one non-neurological organ system, with a significant percentage progressing to organ system failure [4]. Respiratory dysfunction was the most common, followed by cardiovascular dysfunction, coagulation disorders, and renal and hepatic complications. Importantly, organ dysfunction and failure are independently associated with increased in-hospital mortality and worse neurological outcome in those that survive. The mortality rate in patients with no non-neurological organ system failure was 26%, rising to 40% and 47% in those with single-system and two-system failure, respectively. Among 98 patients with severe TBI who went on to become organ donors [13], over 90% had severe cardiac dysfunction or failure, 50% had mild or moderate renal dysfunction, and over 30% had severe pulmonary dysfunction or failure. In a recent study from our institution, we examined 373 patients with severe TBI who progressed to death by neurological criteria [14]. The incidence of systemic complications from this series is seen in **Table 1**.

One very plausible explanation for these variable outcomes is that each individual possesses a multitude of variable gene expression cascades that ultimately affect the functional responses of cells and organ systems to life-threatening injuries. There is some evidence that variants of specific genes present in both the nuclear and mitochondrial genomes are associated with poor outcomes following TBI [15-17]. Other studies have detected genotypic differences in inflammatory cytokine levels that appear causally linked to the development of organ dysfunction following sepsis [18]. However, the relationships between human gene expression and clinical consequences following TBI are largely unknown**.**

Table 1: Incidence of physiologic changes in brain death (from [14]).

Physiologic Abnormality	Incidence (%)
Myocardial Injury	91.4
Hypotension	91.5
Acidosis	79.3
Thrombocytopenia	79.0
Hypothermia	64.4
Moderate-Severe ARDS	61.7
Coagulopathy	61.3
Pancreatic dysfunction	51.4
Diabetes Insipidus	50.1
Hyperglycemia	36.0
Hyperbilirubinemia	18.9
Left ventricular dysfunction	11.7
Kidney dysfunction	9.3

The survival and proper functioning of most cells in the body is dependent on aerobic energy metabolism, which generates 10 times more useful energy in the form of adenosine triphosphate (ATP) than anaerobic energy metabolism. Now many labs, including ours, use the Agilent Seahorse Bioscience extracellular flux analyzer, which can measure both OCR and lactate production via ECAR using only 1% of the cells necessary for use in the oxygen electrode apparatus [19]. One of the most powerful applications of this technology is its applicability to human blood cells, and particularly to platelets and PBMCs. Such measurements are being used as diagnostic biomarkers for mitochondrial diseases and for diseases that are not normally considered to affect blood cells. This concept presents the possibility that circulating blood cells other than erythrocytes can sense metabolic stress in patients and serve as biomarkers of mitochondrial dysfunction in human pathologies, such as diabetes, cardiovascular disease, trauma, and sepsis. Based on recent findings that blood cell energy metabolism reflects that of other cells and tissues present throughout the body [20, 21], advanced cell respirometry measurements performed with blood cells could be particularly helpful for designing precision treatment plans for trauma patients.

Finally, other mitochondrial-based disorders are a consequence of exposure to environmental toxins, e.g., pesticides, or to toxic systemic conditions, e.g., systemic inflammation. In addition, mitochondrial bioenergetics is impaired by direct exposure of cells to bacterial endotoxins [22] and inflammatory cytokines, e.g., interleukin 6 (IL-6) and tumor necrosis factor alpha (TNFα) [23]. By creating a repository of patient samples, we can explore physiological responses beyond the original scope of this study, including proteomic, metabolomic, and cytokine measurements.

Specific Aims:

Severely injured patients, such as those with TBI, who appear otherwise equivalent upon admission, will classically dichotomize into those who survive without life-threatening complications and those who develop uncontrollable complications, such as organ failure and/or sepsis for unclear reasons. The relationships between gene expression, protein expression, cellular bioenergetics, and clinical consequences are, at present, unknown but could be invaluable in the personalized care of critically ill trauma patients.

Aim 1. Determine the temporal relationship between changes in the human blood cell transcriptome and the onset of organ dysfunction and/or sepsis in TBI intensive care unit (ICU) patients.

Aim 2. Advance our understanding of the human gut microbiome and the risk of developing organ dysfunction and sepsis in TBI ICU patients.

Aim 3. Determine the temporal relationship between blood cell energy metabolism and the risk of developing organ dysfunction and sepsis in TBI ICU patients.
Aim 4. Generate a repository of human blood samples with associated clinical data from TBI ICU patients for future additional genomic, proteomic, and metabolomic analyses that will identify molecular biomarkers that associate with development of sepsis or organ dysfunction.
Aim 5. Utilize machine learning techniques and patient data sets to generate predictive models of likelihood of clinical complications soon after hospital admission.

These proposed Aims and hypotheses address the following FY18 Joint Program Committee-6 (JPC-6) Combat Casualty Care Research Program (CCCRP) Precision Trauma Care Research Award (PTCRA) Focus Areas:
Focus Area 1: Improving the Characterization of TBI
Through this study we hope to contribute to the identification and/or characterization of TBI biomarkers that are specific to TBI alone and/or TBI with concomitant injuries (e.g., burn, hemorrhage) and identification of biomarker profile specific to TBI pathology and treatment effectiveness. We also hope to improve the understanding of the role of genetic/genomic factors and/or physiology status on short- and long-term consequences of combat-related TBI.
Focus Area 2: Understanding the factors that influence and/or inform patient responsiveness to TBI therapeutic interventions.
A major objective of this project is to identify and characterize such relationships with the ultimate goal of using this information to provide more effective, personalized care of critically ill trauma patients. We aim to improve understanding of the potential role of differences in TBI treatment decisions and TBI treatment response and to identify biomarkers that inform treatment effectiveness and stage of recovery.

3.0 METHODS

The Trauma Genomics study was approved by the University of Maryland, Baltimore Institutional Review Board (IRB) (Protocol number HP-00080691) and the United States Air Force IRB. The United States Air Force IRB, deferred to University of Maryland, Baltimore IRB to be IRB of record.

3.1 Patient recruitment

A total of 45 patients with a diagnosis of severe TBI and clinically indicated to be admitted to the University of Maryland Shock Trauma Center (STC) Neurotrauma intensive care unit (NTICU) or the Medical intensive care unit (MTICU) were recruited over a period of approximately 3 years. A total of 514 potential participants were screened upon arrival in the Trauma Resuscitation Unit (TRU) and approached by Shock, Trauma, and Anesthesiology Research Center (STAR) Staff (i.e., STAR Staff). See Consort Diagram for details about screening and eligibility **[Figure S1]**. We had a waiver of Health Insurance Portability and Accountability Act (HIPAA) Authorization for purposes of screening medical records to determine which patients appear to meet the eligibility criteria (i.e., diagnosis of severe TBI via presence of intracranial pathology seen on head CT and clinically indicated to be admitted to NTICU or MTICU). The STAR team used a computer

program called the Research Management System (RMS) that automatically filtered out potential study participants based on inclusion and exclusion criteria:
Inclusion Criteria:

- Adults ages ≥ 18 years old
- Presence of intracranial traumatic pathology on head CT
- Clinically indicated to be admitted to the University of Maryland Shock Trauma MTICU or NTICU
- Can speak and understand English

Exclusion Criteria:

- Determination of non-survivability on admission by the neurosurgical team
- Transferred from another hospital more than 24 hours after head injury
- Hemodynamically unstable
- Weight <110 pounds (lbs.)
- Currently incarcerated (i.e., prisoners)
- Pregnant

Once eligibility was determined, STAR research staff explained the study to the patient or the patient's Legally Authorized Representative (LAR) and attempted to obtain informed consent. Due to the life-threatening nature of their illness, most patients were not able to consent for themselves. If possible (awake and alert), potential participants were asked to complete the "Evaluation to Sign Consent" form. If they passed this evaluation, then they could sign and date the informed consent document. In the event the patient did not have the capability to provide informed consent, we attempted to identify and obtain consent from the LAR. The patient or patient's LAR was approached as soon as feasible. If the patient or LAR gave consent for participation, the patient was enrolled in the study. The University of Maryland, Baltimore IRB approved "LAR Identification Form" was completed at that time. Due to the critical nature of collecting samples in the first 48 hours after admission, if the research staff was unable to identify or get access to the LAR, we had a waiver of consent to collect the samples in the first 48 hours per protocol. If the patient or LAR consented in the first 48 hours, we used the collected samples. All other samples from ineligible or non-consented patients were destroyed. If the patient regained capability to sign consent based on the Evaluation to Sign Consent form, after the LAR signed consent, then the patient was consented in order to continue with the study.

3.2 Clinical Measurements, Determination of Sepsis and/or Organ Failure

Sepsis was identified using the definitions described in the Third International Consensus Definitions for Sepsis and Septic Shock [5]. Patients had to have an established infection and a sequential organ failure assessment (SOFA) score greater than or equal to 2. The SOFA score was calculated using the worst values over 24 hours, from the point of the confirmed infection (i.e., time culture that eventually became positive was drawn) [24]. Organ failures were defined using the criteria established according to the SOFA score. SOFA score mortality prediction thresholds were assigned as previously described [25]. The presence of infection was established using a retrospective chart review that encompassed each patient's entire length of stay at the trauma center. Injury severity score (ISS) was calculated using an online calculator, based the original work by Baker et al. [26] (https://qxmd.com/calculate/calculator_356/injury-severity-score).

For the purposes of this study, we compared participants with SOF versus those with neither sepsis nor organ failure (non-SOF) that occurred anytime during the 30-day admission period. Health information was collected from the medical record from admission until discharge (up to 30 days max) including demographics, injury severity, procedures, test results, laboratory values, diagnoses, vital signs, medications, length of stay, number of days in the ICU, ventilator status, and health history to be used as potential covariates.

3.3 Laboratory Methods

3.3.1 Seahorse Preparation, Blood Cell Isolation, and Bioenergetics Measurements

Seahorse preparation: Seahorse XFe24 cell culture plates were coated with Cell-Tak (Thermo Fisher, MA) to facilitate adhesion of cells prior to seeding. A solution of Cell-Tak, diH_2O, and sodium bicarbonate was made according to manufacturer's instructions and 2.4-2.6 micrograms (μg) Cell-Tak was applied to the bottom of each well. The plate was incubated at room temp for 30 minutes (min) before the Cell-Tak solution was aspirated and each well washed twice with PBS. After the second wash, the plates were sealed and kept at 4 degree Celsius (°C) until use. Seahorse XFe24 FluxPak cartridges were hydrated with cell-culture grade water and kept at 37°C overnight. The water was replaced with the supplied Seahorse calibrant solution for at least 3 hours at 37°C before use.

Cell culture reagent preparation: Respirometry and acidification measurements were performed using Dulbecco's Modified Eagle's Medium (DMEM; Sigma-Aldrich, MO) supplemented with 5.5 millimolar (mM) glucose, 4 mM glutamine, 6.2 mM Ethylenediaminetetraacetic Acid (EDTA), 1 mM pyruvate, and an additional 143 mM sodium chloride (NaCl), potential hydrogen (pH) 7.4.

Platelet and PBMC isolation from whole blood: At most 12 ml of whole blood was drawn into K_2-EDTA tubes in the early morning on days 1, 2, 4, and 7 post-injury. The blood tubes were kept on a rocker at room temperature until processing, which started at most 6 hours after blood was drawn. All centrifuge steps were performed at room temperature with no brake during deceleration. Blood was centrifuged at 400 times gravity (x g) for 10 minutes to separate platelet rich plasma from the buffy coat and red blood cells. Plasma was removed and placed in a tube that was centrifuged at 1600 x g for 10 minutes to pellet the platelets. The supernatant platelet poor plasma was removed and stored at -80°C. The platelet pellet was resuspended in DMEM and counted via hemocytometer. PBMCs were isolated from the remaining blood following manufacturer's instructions for the EasySep Direct Human PBMC Isolation Kit (StemCell Technologies, MA). In short, blood was brought back to original volume using PBS (supplemented with 2% Fetal bovine serum (FBS) and 1 mM EDTA) before the addition of the PBMC isolation cocktail, incubation at room temperature, and addition of magnets beads for negative immunomagnetic separation using the supplied magnet for 5 min. The non-magnetized liquid was transferred to a new tube and another dose of beads was added to the new tube for 5 min. After this round of separation, the non-magnetized liquid was again transferred to a new tube and replaced onto the magnet for 5 min for pickup of residual beads. This final liquid was collected and centrifuged at 1200 x g for 10 min. The resulting PBMC pellet was resuspended in DMEM and counted via trypan blue exclusion using a hemocytometer.

Cell plating: 40 million platelets and 500 thousand PBMCs were seeded in their respective Seahorse plate wells in a total of 200 microliters (µl) DMEM. 2 background wells for each cell type were also created using only DMEM. The plate was incubated at 37°C for 30 minutes, and then centrifuged twice at 200 x g for 5 min. The final DMEM volume in each well was brought up to 675 µl and the plate was incubated again at 37°C for 45 minutes.

Seahorse protocol: The Seahorse XFe24 allows for up to 4 injections into each well during the measurement period. The protocol for this study is an adaptation of the Mito Stress Test Assay (Agilent, CA) (**Figure S2**). In order, injections of oligomycin (final concentration 2.5 micromolar (µM)), 2,4-dinitrophenol twice (final concentrations of 30 and 60 µM in each well for platelets, 120 and 160 µM final concentrations for PBMCs), and antimycin A (final concentration 10 µM) were performed to acquire leak respiration, maximum uncoupled respiration, and non-mitochondrial oxygen consumption, respectively. Measurements of OCR and ECAR were performed at multiple time points during the run **[for full protocol, see Table S2].** Individual wells were treated as technical replicates, with the final OCR and ECAR values representing the means of 3-10 wells for each cell type during each time point after outliers and failed wells were removed.

Statistical Analysis: Several types of analyses were performed using Sigmaplot (v 15.0, Systat Software Inc, CA). To examine differences in 1 days post injury (dpi) bioenergetics values, t-tests were used to compare SOF and nonSOF groups. For all other analyses, patients were separated into 3 potential groups: nonSOF, SOF, or prognostic sepsis and/or organ failure (ProgSOF). The ProgSOF group consisted of SOF patients that had any sampling before the SOF diagnosis or who were diagnosed with SOF within 10 days after admission. Samples from all timepoints were pooled into their respective patient groups and were analyzed with a t-test to compare SOF or ProgSOF to the nonSOF group. Finally, in order to compare trends across time, linear regression slopes were determined through Microsoft Excel and a t-test was used to compare groups in Sigmaplot. For slope analysis, only patients with 3 or more sampling time points were included.

3.3.2 Cytokine Measurements

Cytokine measurements were performed on platelet poor plasma samples collected during cell isolation. Samples were kept frozen until analysis via Luminex assays according to manufacturer's instructions (R&D Systems, MN). The sample plate was read using a Luminex MagPix reader and the data was calculated using Luminex's Exponent Software.

Statistical Analysis: See Statistical Analysis for cellular bioenergetics measurements. Since the cytokine measurements were performed on samples that mirrored the cellular bioenergetics sampling, the same analyses were performed.

3.3.3 Blood Cell RNA Collection and Nanostring Gene Expression Analysis

RNA Isolation: 3 ml of whole blood was drawn into Tempus™ blood RNA tubes (ThermoFisher) upon admission to the Shock Trauma hospital and then again during morning rounds on days 1, 2, 4, and 7 post-injury. Tempus tubes were initially kept at room temperature before being stored at 4°C for at most 7 days until storage at -20 °C before processing. RNA was isolated from Tempus tube blood using the Tempus spin RNA isolation kit (ThermoFisher) according to manufacturer's

instructions. Sample quality was accessed using a NanoChip assay on the Agilent Bioanalyzer. The chip was loaded using the manufacturer's protocol. RNA quantity and quality was scored in the 2100 Expert Software. All samples had an RNA Integrity Number (RIN) value between 6.5 and 9. RNA was stored at -80°C until Nanostring analysis.

Nanostring preparation: A 800-gene Nanostring panel was constructed and customized to include genes involved in inflammation, immunity, oxidative stress, hemostasis, and other pathways (Nanostring Technologies, WA). Samples were analyzed for gene expression using Nanostring's hybridization protocol. The manufacturer's protocol was followed for all samples using an input from 30-50 nanograms (ng) of total RNA. All samples were hybridized for a minimum of 17 hours prior to loading the Nanostring prep station followed by imaging on the Nanostring nCounter platform.

Advanced Analysis of Transcriptomics Data: Normalized data was generated using the NanoString nSolver software. All Changes in gene expression are relative to admission (0 dpi) samples which were collected for all patients. All statistical analyses were performed using R (version 4.0.3, R Foundation for Statistical Computing, Austria). An adjusted p-value of <0.05 was considered statistically significant.

3.4 Computer Modeling and Analysis

Logistic Regression Model: To analyze the predictive capabilities of our dataset, the outcome of sepsis/organ failure was modeled by logistic regression using both clinical and gene expression values collected upon or soon after hospital admission [27]. In this model, the Leave One Out Cross-Validation (LOOCV) Variable Selection was implemented. By controlling the number of variables selected for the model and employing internal validation, we kept the model manageable and were able to provide a signature that could be clinically useful while decreasing the potential for overfitting. The LOOCV approach has the advantages of producing model estimates with less bias and more ease in smaller samples. To create the leave-one-out training sets, the SAS software Survey select procedure was used for creating 45 independent replicates of the original data set. Each set (replicate) was identified by a value i where i = 1, …, 45. Individual observations were systematically removed from each of the sets, creating a training set and testing set. We performed the variable selection on training sets 1 through 45, using a logistic model and the best subset selection method in SAS software's procedure.

Extreme Gradient Boosted Tree Model: Extreme gradient boosted (XGB) tree is a type of ensemble learning method that combines multiple decision trees to improve the accuracy of the model. Boosting is an iterative process that adjusts the weights of the observations based on the previous classification results. The algorithm then builds a new decision tree based on the updated weights. Given over 800 variables and 45 cases, we considered selecting variables before model training, which was to reduce overfitting and achieve more stable models. The predictor variables for this model were selected via maximum relevance minimum redundance (MRMR). MRMR is a feature selection algorithm that selects a subset of features that are most relevant to the target variable while minimizing the redundancy between the selected features. It is a non-parametric algorithm, which means that it does not make any assumptions about the distribution of the data. This makes MRMR a more robust algorithm than methods that make assumptions about the data

distribution. It is a filter-based algorithm, which means that it selects features before the machine learning model is trained. This can help to improve the performance of the machine learning model by reducing the number of features that need to be processed. Because there were limited numbers of cases, we selected the top 20 variables using MRMR. A LASSO model was also developed that gave similar results **[Figures S3 and S4].**

4.0 RESULTS

4.1 Cellular Bioenergetics

Overall, platelets did not show significantly different levels of cellular bioenergetics measurements in correlation with SOF diagnosis, although there were some exceptions. Leak respiration was significantly lower in SOF patients at 1 dpi (**Table 2**), although that trend did not hold beyond 1 dpi (**Table 3**). Slope analysis, on the other hand, showed that several bioenergetics variables displayed different trends compared to the nonSOF patients (**Table 4**). With the exceptions of Max OCR and spare capacity, values on average trended upward. However, in regard to almost every metric, the trend upward was significantly greater in the SOF and ProgSOF groups.

In contrast, PBMCs showed a consistent relationship between SOF status and ECAR. At 1 dpi, basal and max ECAR were significantly higher in the PBMCs of SOF patients (**Table 5**), and that trend continued in both the SOF and ProgSOF groups (**Table 6**). We would have expected this to be associated with decreased mitochondrial oxygen consumption, but basal OCR was not affected, and max OCR was in fact higher in both the SOF and ProgSOF groups. Finally, basal ECAR was shown to have a significantly greater trend upward in both the SOF and ProgSOF groups. Taken together, these data imply that the PBMCs of SOF patients were more glycolytic upon admission than their nonSOF counterparts and the ECAR remained elevated throughout sampling.

Table 2: Investigation of platelet bioenergetics and potential correlation to SOF at 1 dpi. A t-test was used to determine significant difference in the comparison of platelet bioenergetics parameters in SOF patients (n=15) and non-SOF patients (n=25) on the first morning after hospital admission. Units are pmol/min for OCR, millipH per minute (mpH/min) for ECAR. For almost all comparisons, the Shapiro-Wilk test for normality failed, so the Mann-Whitney Rank Sum Test was used. †The only exception was the Max ECAR value with means listed instead of medians. *Indicates significance ($p \leq 0.05$).

Measurement	Medians or †Means (non-SOF/SOF)	p-value
Basal OCR	256.1/231.1	0.214
Leak OCR	68.1/54.5	0.049*
Max OCR	525.9/599.8	0.645
Spare Capacity	306.6/302.8	0.922
ATP production	184.3/160.6	0.410
Basal ECAR	58.6/43.2	0.167
Max ECAR†	152.7/133.8	0.270

Table 3: Investigation of platelet bioenergetics and SOF or ProgSOF through all time points. t-tests were used to examine the relationship between platelet bioenergetics and the occurrence of SOF or ProgSOF. Units are pmol/min for OCR, mpH/min for ECAR. The number of samples for each group was different: n=94 for the non-SOF group, n=57 for the SOF group, and n=38 for the ProgSOF group. Almost all comparisons failed the Shapiro-Wilk normality test, therefore the Mann-Whitney Rank Sum test was used with medians provided. †The only exception was Max ECAR, for which means are listed. *Indicates significance ($p \leq 0.05$).

Measurement	Medians or Means† (Non-SOF/SOF/ProgSOF)	p-value (SOF)	p-value (ProgSOF)
Basal OCR	282.5/273.9/279.5	0.693	0.904
Leak OCR	69.4/66.0/67.1	0.185	0.469
Max OCR	602.2/605.2/625.5	0.443	0.275
Spare Capacity	306.4/349.3/359.0	0.259	0.162
ATP Production	214.5/199.5/203.3	0.818	0.976
Basal ECAR	71.3/63.3/65.4	0.517	0.523
Max ECAR†	162.4/161.4/163.0	0.92	0.957

Table 4: Slope analysis of platelet bioenergetics across time to determine relationship to sepsis/organ failure diagnosis. Slope analysis was used to analyze any correlation between trends of platelet OCR and ECAR overtime and occurrence of SOF. The number of samples for each group was different: n=24 for the non-SOF group, n=14 for the SOF group, and n=10 for the prognostic SOF group (ProgSOF). ‡Some comparisons failed the Shapiro-Wilk test for normality, so the Mann-Whitney Rank Sum Test was used with medians provided. *Indicates significance ($p \leq 0.05$).

Measurement	Means or ‡Medians (Non-SOF/SOF/ProgSOF)	p-value (SOF)	p-value (ProgSOF)
Basal OCR	5.6/34.2/25.9	0.001*	0.023*
Leak OCR‡	0.994/4.505/3.22	0.037*	0.151
Max OCR	-12.6/36.2 (SOF) ‡ -14.3/34.6 (ProgSOF)	0.004*	0.007*
Spare Capacity	-16.2/18.3 (SOF) ‡ -14.9/7.6 (ProgSOF)	0.146	0.134
ATP Production	7.22/31.7/24.5	0.001*	0.025*
Basal ECAR	3.2/9.0/6.8	0.006*	0.08
Max ECAR	0.3/11.3/8.6	0.001*	0.016*

Table 5: Investigation of PBMC bioenergetics and potential correlation to SOF at 1 dpi. A t-test was used to determine significant difference in the comparison of PBMC bioenergetics parameters in SOF patients (n=15) and non-SOF patients (n=25) on the first morning after hospital admission. Units are pmol/min for OCR, mpH/min for ECAR. *Indicates significance ($p \leq 0.05$).

Measurement	Means (non-SOF/SOF)	p-value
Basal OCR	132.5/143.2	0.471
Leak OCR	41.9/37.8	0.249
Max OCR	242.5/285.4	0.084
Spare Capacity	80.1/131.9	0.282
ATP production	91.0/89.9	0.379
Basal ECAR	22.5/29.3	0.015*
Max ECAR	38.449/49.5	0.019*

Table 6: Investigation of PBMC bioenergetics and SOF or ProgSOF through all time points. t-tests were used to examine the relationship between PBMC bioenergetics and the occurrence of SOF or ProgSOF. The number of samples for each group was different: n=91 for the non-SOF group, n=57 for the SOF group, and n=39 for the ProgSOF group. Units are pmol/min for OCR, mpH/min for ECAR. All comparisons failed the Shapiro-Wilk normality test, so the Mann-Whitney Rank Sum test was used with medians provided. *Indicates significance ($p \leq 0.05$).

Measurement	Medians Non-SOF/SOF/ProgSOF	p-value (SOF)	p-value (ProgSOF)
Basal OCR	124.7/121.3/120.0	0.836	0.603
Leak OCR	41.6/37.9/35.2	0.051	0.015*
Max OCR	248.1/272.2/272.0	0.022*	0.054
Spare Capacity	115.7/142.7/148.9	0.064	0.053
ATP Production	89.2/86.0/80.5	0.455	0.953
Basal ECAR	23.3/32.5/32.5	<0.001*	<0.001*
Max ECAR	40.5/49.4/49.4	0.002*	0.002*

Table 7: Slope analysis of PBMC bioenergetics across time to determine relationship to sepsis/organ failure diagnosis. Slope analysis was used to analyze any correlation between trends of PBMC OCR and ECAR overtime and occurrence of SOF. The number of samples for each group was different: n=23 for the non-SOF group, n=13 for the SOF group, and n=10 for the prognostic SOF group (ProgSOF). ‡Several comparisons failed the Shapiro-Wilk test for normality, so the Mann-Whitney Rank Sum Test was used with medians provided. *Indicates significance ($p \leq 0.05$).

Measurement	Means or ‡Medians (Non-SOF/SOF/ProgSOF)	p-value (SOF)	p-value (ProgSOF)
Basal OCR	-6.0/-1.7 (SOF) ‡ -2.7/-2.8 (ProgSOF)	0.754	0.971
Leak OCR‡	-0.6/-0.6/-0.9	0.882	0.784
Max OCR‡	-5.5/0.3/0.9	0.729	0.695
Spare Capacity‡	0.1/1.3/1.3	0.521	0.557
ATP Production	-4.1/-2.1 (SOF) ‡ -2.7/-3.2 (ProgSOF)	0.705	0.874
Basal ECAR	0.184/2.5/2.6	0.017*	0.012*
Max ECAR	-0.16/1.7 (SOF) ‡ -0.3/2.7 (ProgSOF)	0.193	0.110

4.2 Cytokine Measurements

We originally conducted a pilot study focusing on the platelet poor plasma of only SOF positive patients and 12 individual cytokines: interleukins 1β, 6, 8, 10, 12, and 16 (IL-1β, IL-6, IL-8, IL-10, IL-12, and IL-16 respectively), monocyte chemoattractant protein-1 (MCP-1), TNFα, interferon-ɣ (IFN-ɣ), interferon gamma-induced protein 10 (IP-10/CXCL10), macrophage inflammatory protein 1β (MIP1β), and regulated on activation, normal T cell expressed and secreted (RANTES)/ Chemokine C-C motif ligand 5 (CCL5). Detection for 5 cytokines (IL-1β, IL-12, MCP-1, IFN-ɣ, and MIP1β) was below the detectable range of the kits and these cytokines were omitted from further measurements. The remaining 7 cytokines were measured at each time point for the remaining patients.

At 1 dpi, both IL-6 and IL-16 showed a significant difference between SOF and non-SOF patients, with the SOF group exhibiting higher levels than the non-SOF group for both cytokines (**Table 8**). This continued throughout sampling, as IL-6 and IL-16 maintained higher levels for both SOF and ProgSOF groups (**Table 9**), indicating that increased IL-6 and IL-16 correlate to SOF diagnosis from an early time point and those higher levels are maintained. Meanwhile, IL-8, IP-10, and IL-10 were higher in both the SOF and ProgSOF groups (**Table 9**), indicating increased pro- and anti-inflammatory signaling. Finally, slope analysis showed that in the SOF and ProgSOF groups, TNFα, IL-8, and RANTES trended upward, with significance indicated (**Table 10**).

Table 8: Investigation of cytokine levels and potential correlation to SOF at 1 dpi. A t-test was used to determine the p-values comparing SOF patients (n=15) to non-SOF patients (n=26) on the first morning after hospital admission. All analyses except for RANTES/CCL5 failed the Shapiro-Wilk normality test, so the Mann-Whitney Rank Sum test was used, and medians given. *Indicates significance ($p \leq 0.05$).

Cytokine	Medians (Non-SOF/SOF)	p-value
TNFα (pg/ml)	2.8/2.7	0.797
IL-6 (pg/ml)	24.5/71.2	0.005*
IL-8 (pg/ml)	2.3/3.6	0.493
IP-10 (pg/ml)	21.9/21.4	0.586
IL-10 (pg/ml)	3.0/4.7	0.142
IL-16 (pg/ml)	115.4/189.2	0.016*
RANTES/CCL5 (ng/ml)	8.3/8.2 (means)	0.971

picograms per milliliter (pg/ml); nanograms per milliliter (ng/ml)

Table 9: Investigation of cytokine levels and SOF through all time points. t-tests were used to compare cytokine levels measured in non-SOF (n=94) samples and SOF (n=58) or ProgSOF (n=45) samples. Units are pg/ml except RANTES which is ng/ml. All comparisons failed the Shapiro-Wilk normality test, so the Mann-Whitney Rank Sum test was used with medians provided. *Indicates significance ($p \leq 0.05$).

Cytokine	Medians Non-SOF/SOF/ProgSOF	Results (SOF)	Results (ProgSOF)
TNFα	3.3/4.5/3.2	0.171	0.727
IL-6	17.6/42.3/44.3	<0.001*	<0.001*
IL-8	2.8/3.7/3.7	0.046*	0.096
IP-10	27.6/34.4/38.9	0.005*	0.003*
IL-10	3.1/5.1/5.3	<0.001*	<0.001*
IL-16	96.3/127.2/123.5	<0.001*	0.001*
RANTES	8.9/10.2/10.8	0.441	0.077

Table 10: Slope analysis of cytokine levels across time to determine relationship to SOF diagnosis. Slope analysis was used to analyze any correlation between trends of cytokine levels in circulation overtime and SOF. The number of samples for each group was different: n=23 for the non-SOF group, n=14 for the SOF, and n=10 for the ProgSOF group. Almost all comparisons failed the Shapiro-Wilk normality test, so the Mann-Whitney Rank Sum test was used with medians provided. †The only exception was RANTES, for which means are listed. *Indicates significance ($p \leq 0.05$).

Cytokine	Medians or Means† Non-SOF/SOF/ProgSOF	Results (SOF)	Results (ProgSOF)
TNFα	0.176/0.480/0.438	0.049*	0.137
IL-6	-3.6/-6.8/-6.8	0.364	0.273
IL-8	0.10/0.84/0.67	0.021*	0.082
IP-10	1.74/4.82/2.44	0.183	0.762
IL-10	-0.24/-0.10/-0.15	0.586	0.940
IL-16	-3.6/-6.8/-6.8	0.364	0.273
RANTES†	0.149/0.724/0.948	0.091	0.045*

4.3 Blood Cell Transcriptomics

The initial assessment of differences in gene expression between patients diagnosed with SOF and non-SOF controls at each individual dpi did not result in any significant results. Next, we focused on gene expression changes over time within the non-SOF control and SOF groups using a likelihood ratio test. We identified 47 genes with positive trends and 177 genes with negative trends over time in the non-SOF group and 40 genes with positive trends and 143 genes with negative trends over time in the SOF group. Comparing the trends between the two groups, we identified 3 genes, namely GALNT14, PCOLCE2, and VNN1 that showed positive trends over time in SOF patients while showing negative trends over time in the non-SOF group (**Figure 1**). We then divided the SOF group into 3 subgroups: AdmitSOF patients were diagnosed with SOF upon admission or within 1 day after admission before most sampling was completed, ProgSOF patients are as previously described, and LateSOF patients were diagnosed with SOF after 10 dpi, well after sampling had been completed. These groups accounted for 36, 17, and 39 genes showing a positive trend respectively and 52, 54, and 114 genes showing a negative trend respectively. Comparing the trends between AdmitSOF and NonSOF groups, we identified 11 genes, namely C3AR1, FCGR1A, FOLR3, GALNT14, GRB10, MS4A4A, NAIP, S100A8, S100A12, SLC26A8, and TDRD9 that showed positive trends over time in AdmitSOF patients while showing negative trends NonSOF control group (**Figure 2**). Similarly, comparing the trends between the LateSOF and NonSOF groups, we identified 4 genes, namely GALNT14, OLAH, TDRD9, and VNN1 that showed positive trends over time in LateSOF while showing negative trends over time in the NonSOF group (**Figure 3**).

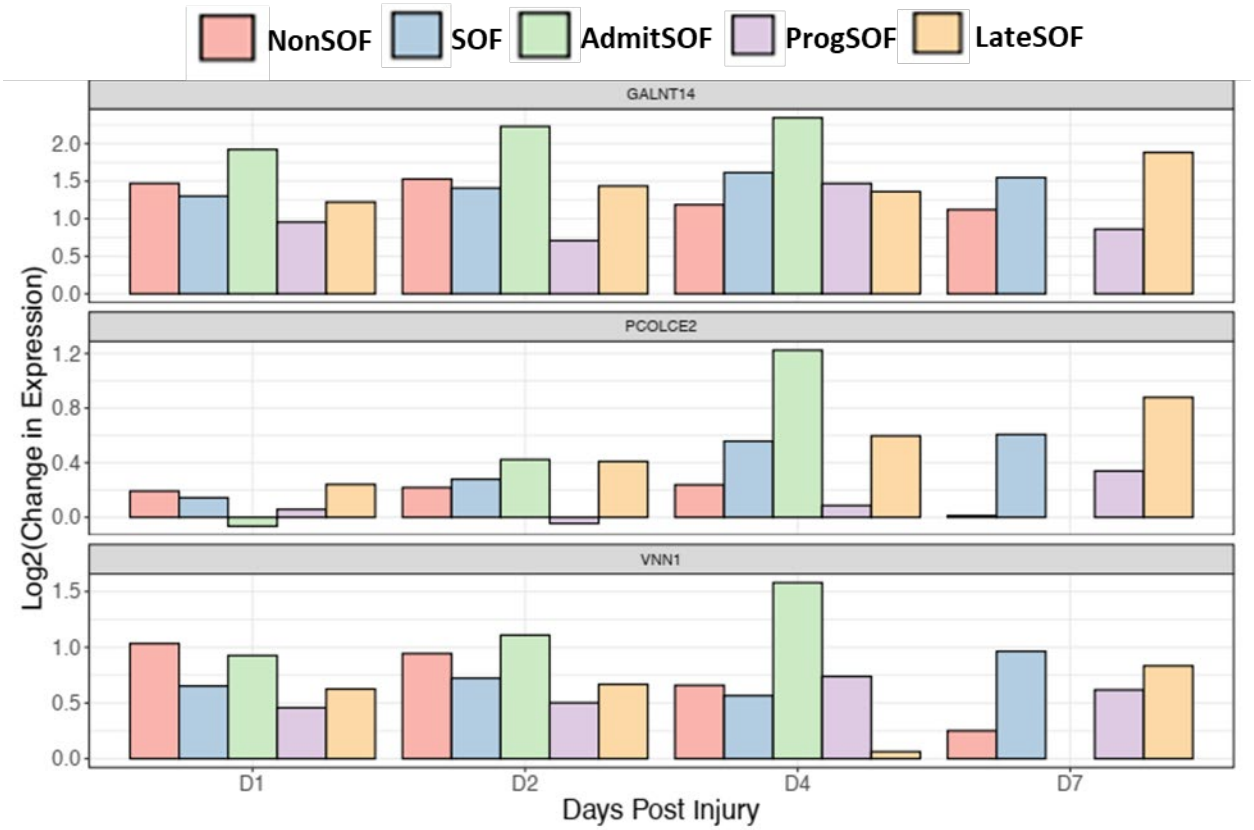

Figure 1: Change in expression of significant genes that showed positive trends in SOF patients and negative trends in NonSOF patients. Plot illustrates the change in expression of candidate genes at different dpi for the NonSOF control group (red), all patients diagnosed with SOF (blue), patients diagnosed with SOF early after admission (AdmitSOF; green), patients diagnosed with SOF during sampling (ProgSOF; purple), and patients diagnosed with SOF after sampling was completed (LateSOF; yellow). All values represent relative changes in expression based on Day 0 (admission) values.

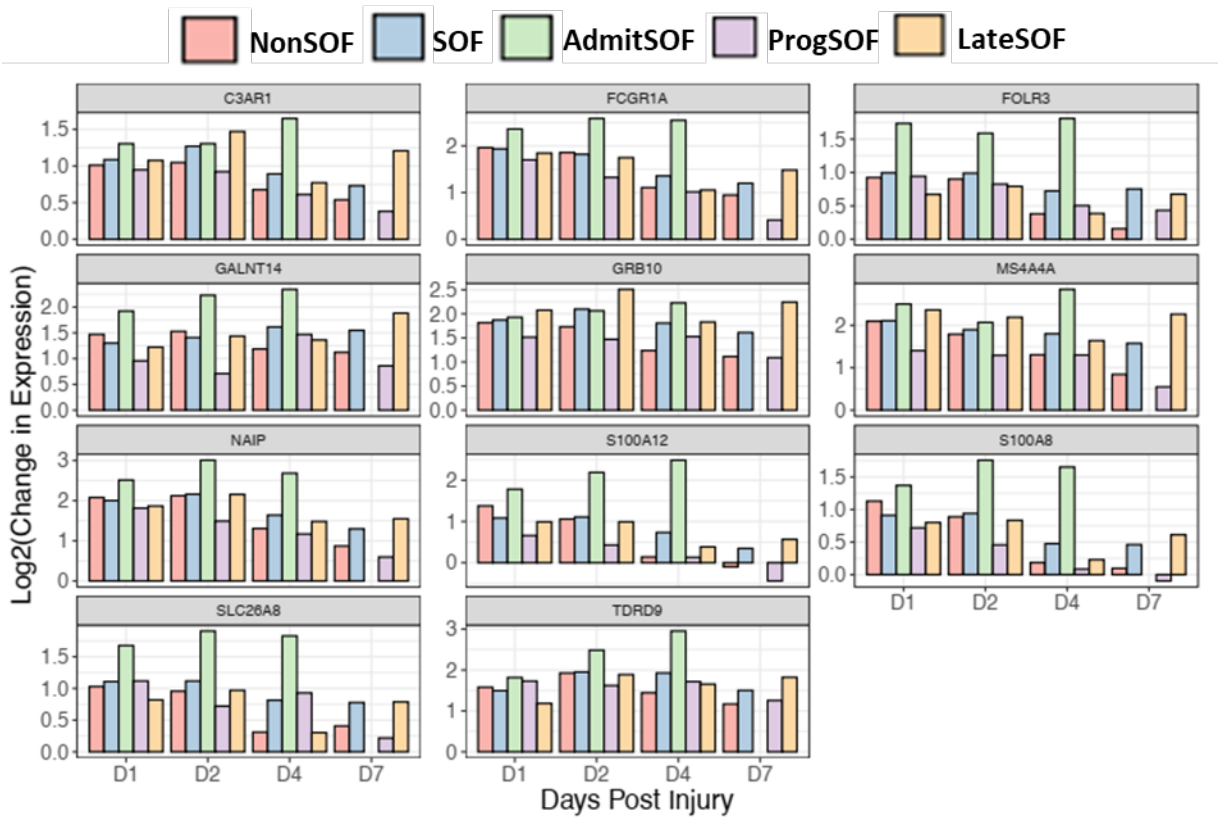

Figure 2: Change in expression of significant genes from the AdmitSOF subgroup over time compared to NonSOF. Plot illustrates the change in expression of candidate genes at different dpi for the NonSOF control group (red), all patients diagnosed with SOF (blue), AdmitSOF (green), ProgSOF (purple), and LateSOF (yellow). All values represent relative changes in expression based on Day 0 (admission) values.

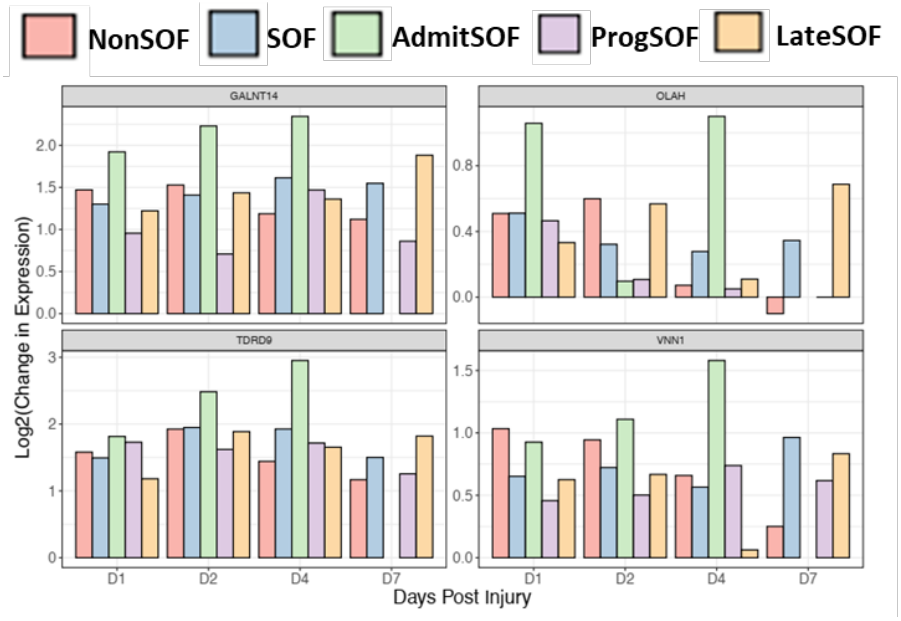

Figure 3: Change in expression of significant genes from the LateSOF subgroup over time. Plot illustrates the change in expression of candidate genes at different dpi for the NonSOF control group (red), all patients diagnosed with SOF (blue), AdmitSOF (green), ProgSOF (purple), and LateSOF (yellow). All values represent relative changes in expression based on Day 0 (admission) values.

4.4 Computer Modeling

4.4.1 Logistic Regression Model

As an initial examination into the prediction capacity for our dataset, we utilized a logistic regression model. The variables used for this model were based on 0 dpi measurements, meaning only measurements taken upon admission. This included clinical variables and gene expression measurements, but it did not cover bioenergetics metrics since the earliest those were measured was at 1 dpi. Specifically, variables were selected based on their significant correlation to SOF status or those that were considered clinically important to the diagnosis. The clinical variables selected were age, lactate, GCS score, heart rate (HR), SBP, temperature, SI, calcium, and PTT. The gene expression variables were selected through a univariate test with a $p \leq 0.05$ and were ATOX1, CYCS, NOD2, IFNg, CFD, CD24, BTK, CEACAM6, CYBB, PTTG1, GFER, and CEACAM8.

The SAS software's selection method allowed us to control the number of variables we wished to include in the model, and in this case, we chose k=4-7, meaning models would be generated using the top 4-7 variables. A variable was selected if it was in 60% – 70% of the models. To avoid the risk of overfitting, the LOOCV of the model for each k was performed and the AUROCs of their cross-validation were calculated, with the 5-variable model performing the best (**Table 11**). A comparison of the models showed no significant difference between them as no p-value was less than 0.05 (**Table 12**), therefore we would select model 5 as the most predictive with gene

predictors ATOX1 and CFD and clinical predictors SBP, calcium, and GCS being the most important. We followed this up with more advanced machine learning analysis.

Table 11: Comparison of logistic regression models based on number of variables.

Model (k)								AUC of the model	AUC of the cross-validation
4	ATOX1	CFD	SBP	calcium				0.9443	0.8782
5	ATOX1	CFD	SBP	calcium	GCS			0.9496	0.9055
6	ATOX1	CFD	SBP	calcium	GCS	CYCS		0.9727	0.8824
7	ATOX1	CFD	SBP	calcium	GCY	CYCS	PTTG1	0.9685	0.8655

Table 12: Comparison of other models to the 5-variable model.

Contrast	Estimate	Standard Error	95% Wald Confidence Limits		Chi-square	Pr > ChiSq (p-value)
4 vs 5	-0.0063	0.0199	-0.0453	0.0327	0.1003	0.7515
6 vs 5	0.0231	0.0274	-0.0306	0.0768	0.711	0.3991
7 vs 5	0.0189	0.0251	-0.0302	0.068	0.5695	0.4505

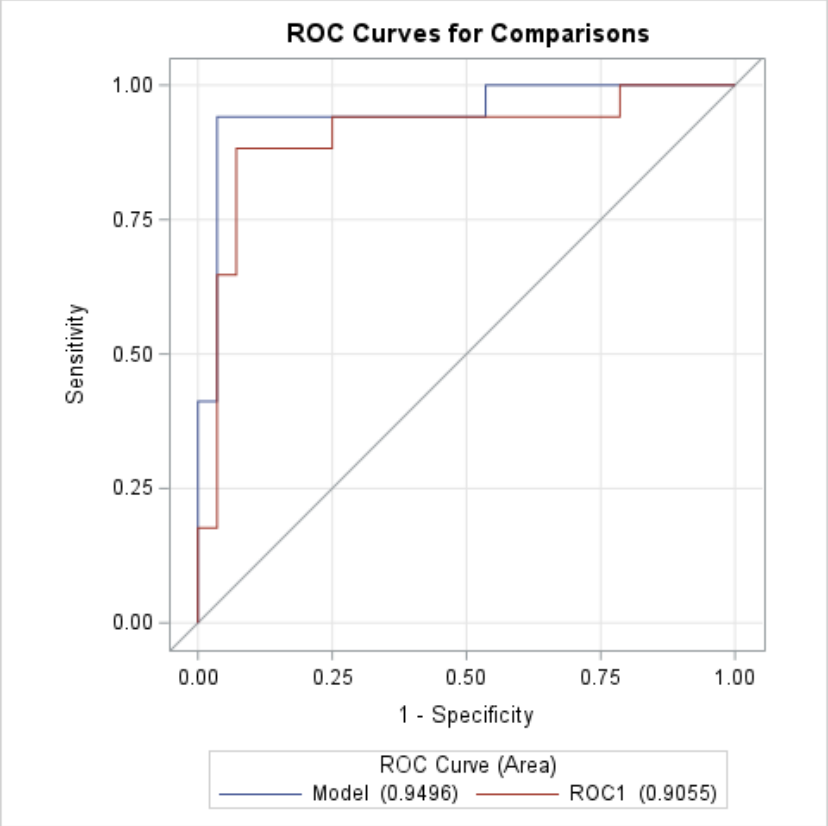

Figure 4: ROC (Receiver operating characteristic) curves of training and testing. This is to evaluate the model's performance on unseen data. The area under the receiver operating characteristic curve is used as the performance metric. The blue curve is for training and the red curve is for testing.

4.4.2 Extreme Gradient Boosted Tree Model

As a result of MRMR variable selection, this model included clinical variables and gene expression values collected upon or soon after admission as well as blood cell bioenergetics values collected at 1 dpi. Clinical variables included ISS, head Abbreviated Injury Score (AIS), age, sex, lactate, HR, SBP, GCS, temperature, calcium, and PTT. Bio-energetic variables included basal and max ECAR for the PBMCs, and gene expression variables were selected from almost 800 individual gene transcript values.

To create machine learning models and validate their predictive performance, especially on unseen new datasets, we used a repeated 10-fold cross-validation method. The dataset was split into 10 folds with stratified sampling, where each fold has a similar proportion of positive outcome. One-fold was used for testing and the remaining folds were used for training and hyper-parameter tuning. To mimic the different combinations of cases, we shuffle the dataset and repeat the above process 10 times and used the averaged results to evaluate each learning algorithm's performance on this dataset. We used the Area Under the Receiver Operating Characteristics curves (AUROCs) and their 95% confidence intervals (CIs) to evaluate the prediction performance (**Figure 5**).

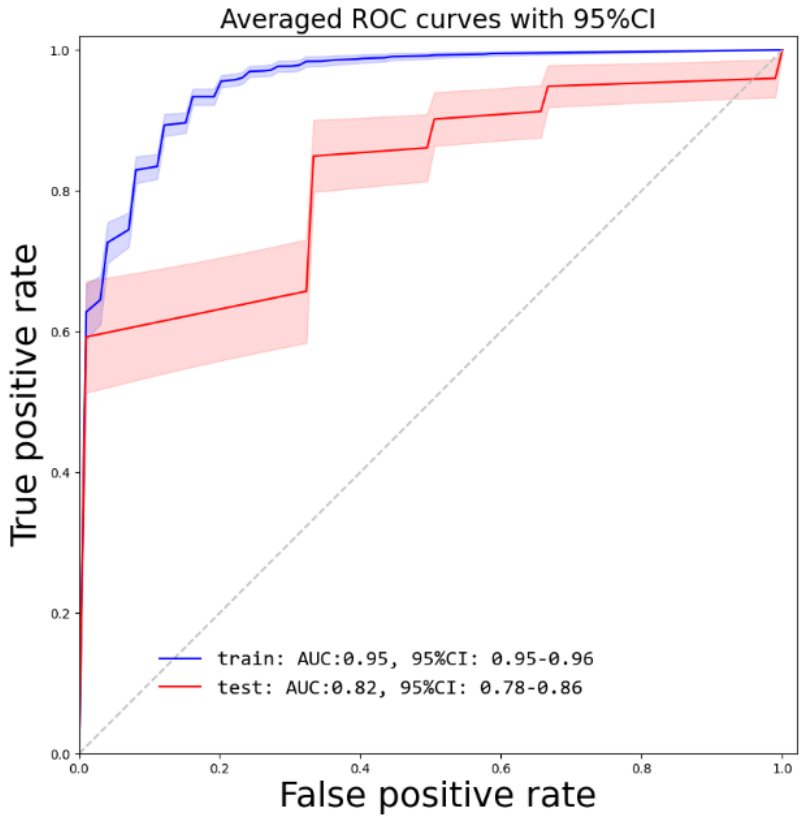

Figure 5: Extreme Gradient Boosting Tree Model. ROC (Receiver operating characteristic) curves of training and testing (10 folder cross-validation repeated 10 times). The testing AUC is 0.82 with 95% CI 0.78 to 0.86, which shows that the XGB model has a reasonable predictive performance on unseen data.

For model interpretation, we analyzed the variables' contribution to the models' prediction to gain insights. The Shapley Additive explanations (SHAP) values were used to calculate the importance of a variable by comparing what a model predicts with and without the variable. [28]. The variable importance at a global level is given by adding the absolute value of the SHAP values for each individual data point. SHAP values were calculated and displayed graphically in order to describe each variable's contribution to the outcome prediction and improve model interpretability. The SHAP values were used to peek into the models, which explain how much each variable contributes to the difference between the actual output and the expected output of a model for a given input data point. Positive SHAP values indicate that a variable increases the model output relative to its expected value, while negative SHAP values indicate that a variable decreases the model output relative to its expected value. The XGB model ranked the most important variables in its prediction model which included clinical (PTT, calcium, SBP), gene expression (BNIP3, GSTM1, NOD2, GNLY, ATF3, and DOCK1), and bioenergetics (PBMC Max ECAR, PBMC Basal ECAR) variables **(Figure 6)**. From an individual metric level, we plotted the top four important variables' values (x axis) against their SHAP values (y axis) to show each variable's impact on the prediction **(Figure 7)**.

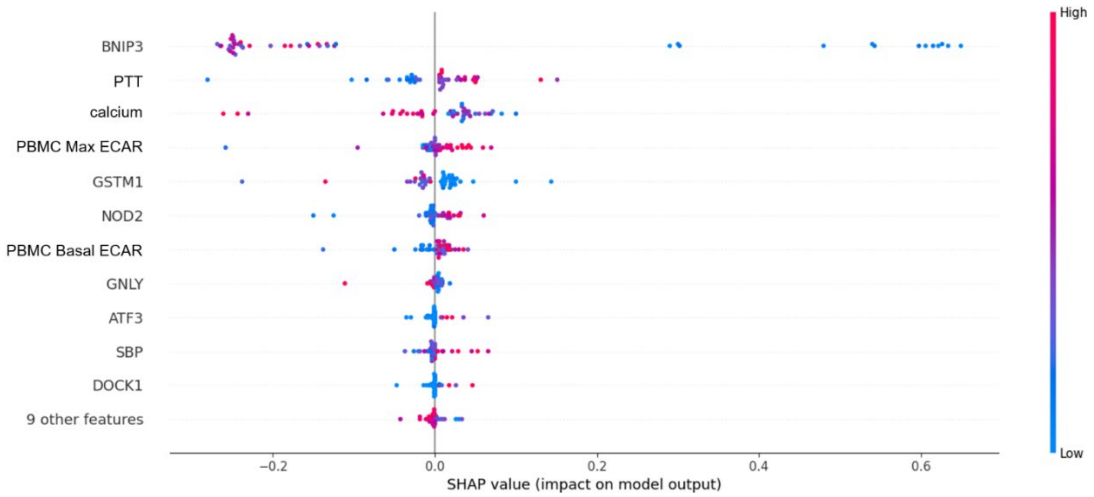

Figure 6: Variable importance ranking via SHAP values for XGB model. The SHAP values are used to explain how much each variable contributes to the difference between the actual output and the expected output of a model for a given input data point. Each dot is one patient's value for a variable. Blue means its value is low and red is high (scale on right). A dot located on the right side means the value of that variable increases the chance of developing SOF, while a dot on the left side means the value of that variable decreases the chance. The variable importance at a global level is given by adding the absolute value of the SHAP values for each individual data point.

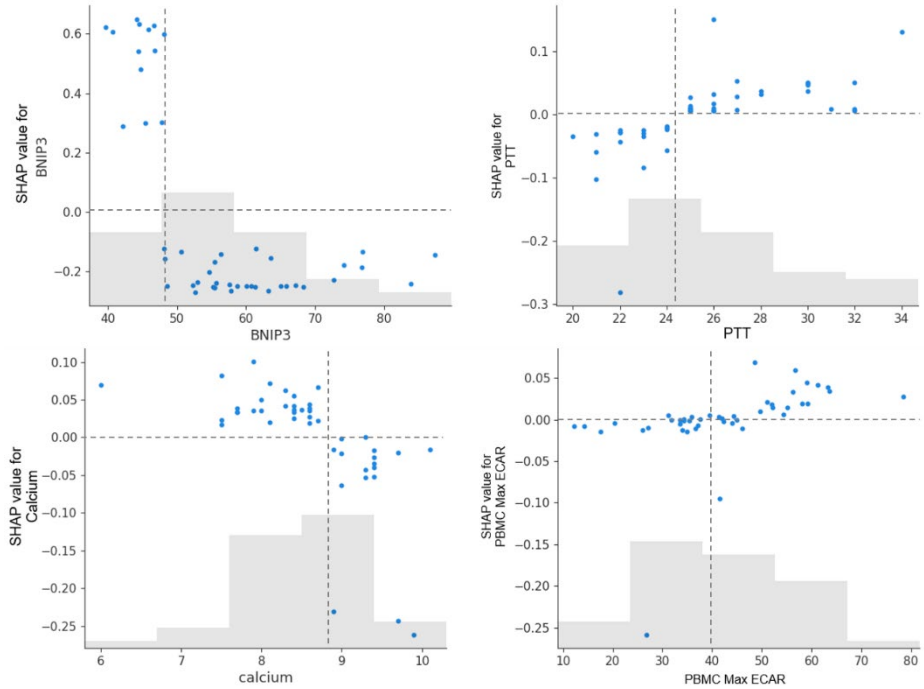

Figure 7: Scatter plots of the top 4 important variables in the XGB model. The spread of the top 4 most important variables of the XGB model are shown: gene expression of BNIP3, clinical variables PTT and calcium, and PBMC Max ECAR. The x-axis represents the scale of the measurements, while the y-axis represents corresponding SHAP values. These plots show the relative impact of the variable on the model's prediction given all other variables being the same.

For example, when the BNIP3 value is above 50, it decreases the chance (log-odds) of having a positive outcome, which in this case is SOF diagnosis. Similarly, when PTT's value is above 24, it increases the chance of having a positive outcome. The gray areas represent histograms showing the distribution of data values.

5.0 DISCUSSION

The power to direct differences in outcomes between patients who were diagnosed with SOF compared to those who had neither is a gap in clinical care that is rightly being addressed. There is a need to understand and recognize biomarkers as prognostic indicators of bad outcomes, either early after admission or during care. While certain clinical metrics are used to diagnose SOF after the fact, this study provides several metrics that may be useful in early detection.

Our cellular bioenergetics analyses revealed that in PBMCs the ECARs were increased in SOF compared to non-SOF patients. While this is not necessarily surprising, as increased glycolysis is often associated with less efficient mitochondria-derived metabolism, we did not observe a corollary decrease in OCR in our samples and in fact we saw an increase in PBMC OCR values in general. Nevertheless, the PBMC ECAR rates associated so strongly with SOF outcome at 1 dpi that the MRMR algorithm independently selected them as important predictive variables. Meanwhile, we measured cytokines from the plasma stored during this process and found several correlations to SOF, primarily with IL-6 and IL-16 but also IL-8, IL-10, and IP-10.

Our analysis of blood cell gene expression also provided valuable insight into the genetic response of PBMCs to SOF. Several genes were differentially expressed between patients who would go on to develop SOF than those that wouldn't. But considering the highly individualized nature of gene expression, the longitudinal analysis of expression over time allowed some normalization between patients and revealed trends of certain genes with SOF diagnosis, the most significant being GALNT14, a protein that is involved in the regulation of inflammation. In sepsis, GALNT14 is overexpressed, which leads to increased inflammation and tissue damage through activation of the NF-κB pathway. Hence, drugs that target GALNT14 could be used to reduce inflammation and tissue damage in patients with complications.

The construction of our expansive dataset, that includes both clinical and laboratory observations, allowed us to utilize advanced computer modeling and machine learning techniques. The XGB tree model concluded that specific metrics in our dataset are reasonably predictive of eventual SOF diagnosis, but this analysis has its limitations. First, we only used the first day observations to predict SOF. The temporal patterns of those variables were not used in this approach. Additionally, we set the MRMR to select the top 20 variables. This number of variables may not be optimal for each learning algorithm. Fine tuning the set of input variables may improve the model's prediction performance and interpretability. Third, although we used 10-fold cross-validation, the data set is of a small size. More training and testing data would enhance the generalizability of the model. However, additional information, such as provided by the cytokine measurements, could add to and further refine the model to be more predictive.

This study was severely limited by the overlapping COVID-19 pandemic. Although COVID diagnosis was not an exclusionary criterion, it considerably decreased the potential patient population through the first two years of the study, as TBI is less common in general during a time of decreased public activity. Although we were still able to recruit 45 patients, 17 of which were diagnosed with SOF, a greater enrolled patient population would give much greater power to our

results. While the initially proposed number of 240 patients would have been beneficial, important information was obtained with our smaller number of enrollees.

Future directions/next steps: We still possess many banked samples that can be used for future work. For most time points we have platelet and PBMC pellets that can be used for further investigation of metabolomics or proteomics. Our banked plasma samples could also be used for proteomics or to measure other cytokines or catecholamines. And our stored RNA could be used for future studies, either on a focused scale with quantitative real-time polymerase chain reaction (qPCR) or a much broader scale using microarrays. Finally, the data we have already generated is ripe for mining. The focus of this study was correlation between our variables and the diagnosis of sepsis/organ failure. However, our variables may correlate with each other in ways we have not yet analyzed. For instance, considering cytokines are an important protein for cell signaling, there could be correlations between cytokines and the expression of certain genes that are as yet unrecognized but can be gleaned from our dataset. Additionally, there are certain gene transcripts that follow similar patterns in the vast majority of patients, regardless of sepsis/organ failure diagnosis. Any break from those patterns could be indicative of a specific response from that patient(s) and could be worth further exploration.

6.0 CONCLUSIONS

We have successfully completed the specific aims of this project. The following milestones and deliverables were achieved:

Aim 1. Determine the temporal relationship between changes in the human blood cell transcriptome and the onset of organ dysfunction and/or sepsis in TBI ICU patients.

We hypothesized that the gene transcription response of PBMC during the first week following TBI can serve as a biomarker that is useful for predicting whether or not patients will experience sepsis or organ failure. We have found several genes that have differential gene expression between TBI patients who experienced SOF and those that did not. The most notable of these for prognostic purposes were GALNT14, PCOLCE2, and VNN1. We also identified other genes of interest that were differentially expressed in smaller subgroups of SOF patients.

Aim 2. Advance our understanding of the human gut microbiome and the risk of developing organ dysfunction and sepsis in TBI ICU patients.

We attempted to collect stool samples at admission and days 1, 2, 4, and 7 of hospitalization on all participants in the Trauma Genomics study. Unexpectedly, rectal exams were not done routinely on admission. This reduced the number of admission stool samples that were collected. We also specified in the protocol that we would only collect stool samples when the participant spontaneously defecated. Many participants were missing stool samples during hospital admission because they did not spontaneously defecate. In addition, despite multiple trainings and reminders from nurse management, the nurses would sometimes forget to collect the stool samples when the participant did have a bowel movement. We consulted with Dr. Claire Fraser, an investigator on this project with expertise in microbiome analysis, and together determined that given the dearth

of samples that were collected, we would not have enough data points to conduct a meaningful microbiome analysis. Therefore, we discontinued the collection of stool samples on the remaining participants. This protocol amendment was approved by the Institutional Review Board on 9/29/2022.

Aim 3. Determine the temporal relationship between blood cell energy metabolism and the risk of developing organ dysfunction and sepsis in TBI ICU patients.

We hypothesized that advanced cell respirometry measurements performed with freshly drawn blood cells would show a relationship between sepsis/organ failure status and cellular bioenergetics. We have shown that in PBMCs, increased ECAR correlated with SOF diagnosis. This was further confirmed via machine learning methods, as both the basal and max ECAR rates were independently chosen by MRMR in XGB model construction as important variables to predict SOF during early-stage care.

Aim 4. Generate a repository of human blood samples with associated clinical data from TBI ICU patients for future additional genomic, proteomic, and metabolomic analyses that will identify molecular biomarkers that associate with development of sepsis or organ dysfunction.

We have banked several samples for each patient at multiple time points. These include aliquots of whole blood RNA, platelet poor plasma, and cell pellets. We have also constructed a database of clinical data from these patients for up to 30 days. In total, these sample and data banks can help further research beyond the scope of this study. For instance, the cytokine measurements on platelet poor plasma samples have already revealed several cytokines that were increased in SOF patients, including IL-6 and IL-16. New types of analyses would add to our extensive data set and could refine predictive models and overall care going forward. Investigators from the Air Force Research Labs and from Air Force funded labs will be given priority for both existing and future data generated by these assays.

Aim 5. Utilize machine learning techniques and patient data sets to generate predictive models of likelihood of clinical complications soon after hospital admission.

Identification of patients at risk for SOF that would allow for early initiation of preventative measures and treatments could be ground-breaking in the care of patients with TBI. We have developed several models based on our data that shows the predictive quality of specific variables, including gene expression, bioenergetics, and clinical metrics, to alert clinicians of potential complications during care, and these models can potentially be further refined with increased data input.

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APPENDIX: GENE LIST

Table S1: List of mentioned genes.

Gene Symbol	Gene Name	NCBI Accession #
ATF3	Activating Transcription Factor 3	NM_001674.3
ATOX1	antioxidant 1 copper chaperone	NM_004045.3
BNIP3	BCL2 interacting protein 3	NM_004052.3
BTK	Bruton agammaglobulinemia tyrosine kinase	NM_000061.1
C3AR1	Complement component 3a receptor 1	NM_004054.2
CD24	CD24 molecule	NM_013230.2
CEACAM6	CEA-related cell adhesion molecule 6	NM_002483.4
CEACAM8	CEA cell adhesion molecule 8	XM_011526341.1
CFD	complement factor D (adipsin)	NM_001928.2
CYBB	cytochrome b-245 beta chain	NM_000397.3
CYCS	cytochrome c, somatic (mitochondrial)	NM_018947.4
DOCK1	dedicator of cytokinesis 1	NM_001290223.1
FCGR1A	Fc fragment of IgG receptor 1A	NM_000566.3
FOLR3	folate receptor 3 (gamma)	NM_000804.2
GALNT14	N-acetylgalactosaminyltransferase 14	NM_001253826.1
GFER	growth factor, augmenter of liver regeneration	NM_005262.2
GNLY	granulysin	NM_012483.3
GRB10	growth factor receptor bound protein 10	NM_001001555.2
GSTM1	glutathione S-transferase M1	NM_000561.2
IFNG	interferon gamma	NM_000619.2
MS4A4A	membrane spanning 4-domains A4A	NM_001243266.1
NAIP	NLR family apoptosis inhibitory protein	NM_004536.2
NOD2	nucleotide-binding oligomerization domain containing 2	NM_022162.1
OLAH	oleoyl-ACP hydrolase	NM_001039702.2
PCOLCE2	procollagen C-endopeptidase enhancer 2	NM_013363.2
PTTG1	pituitary tumor-transforming 1	NM_004219.2
S100A12	S100 calcium binding protein A12	NM_005621.1
S100A8	S100 calcium binding protein A8	NM_002964.4
SLC26A8	solute carrier family 26 member 8	NM_001193476.1
TDRD9	tudor domain containing 9	NM_153046.2
VNN1	vanin 1	NM_004666.1

Supplemental Methods

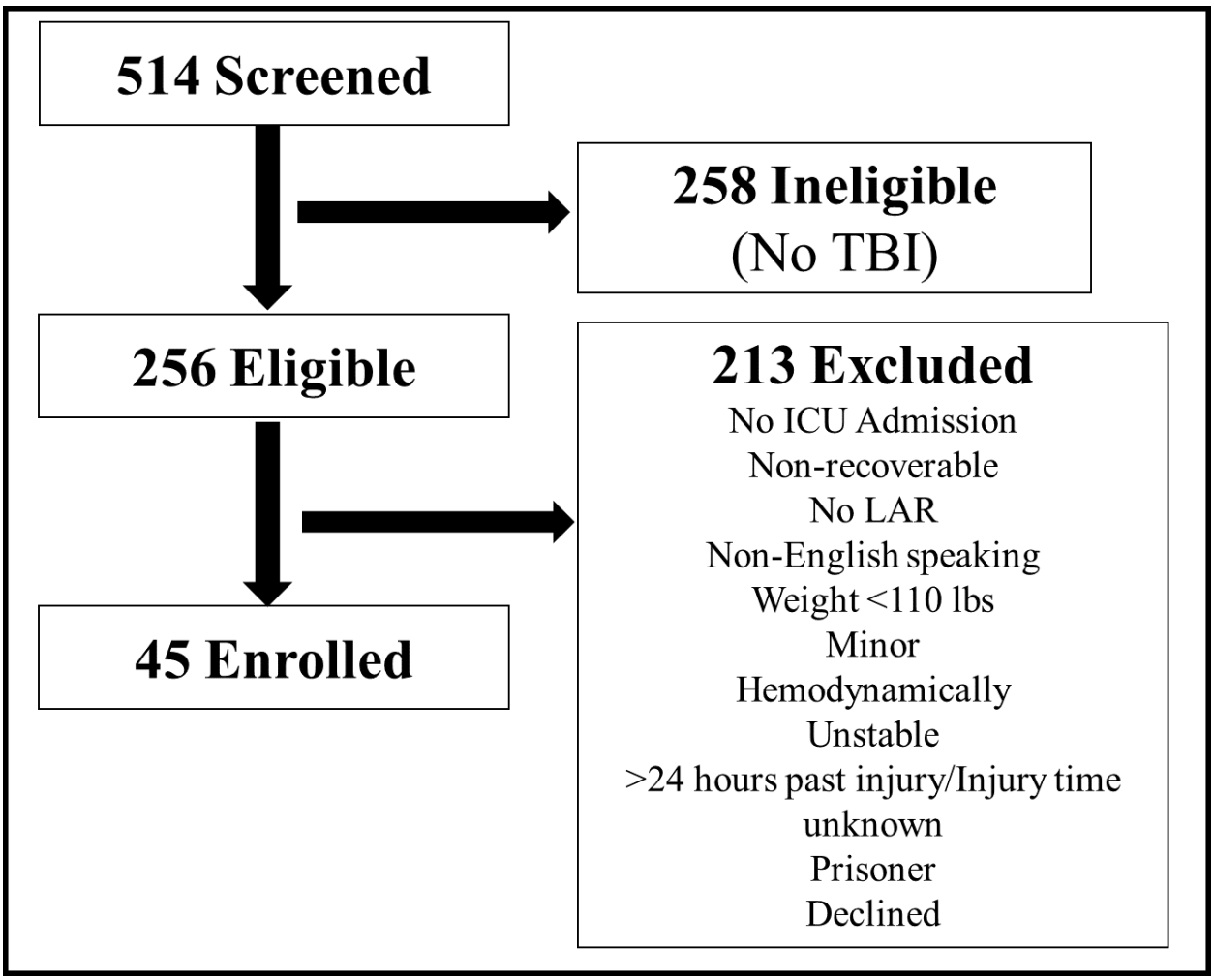

Figure S1: Consort diagram for participant enrollment

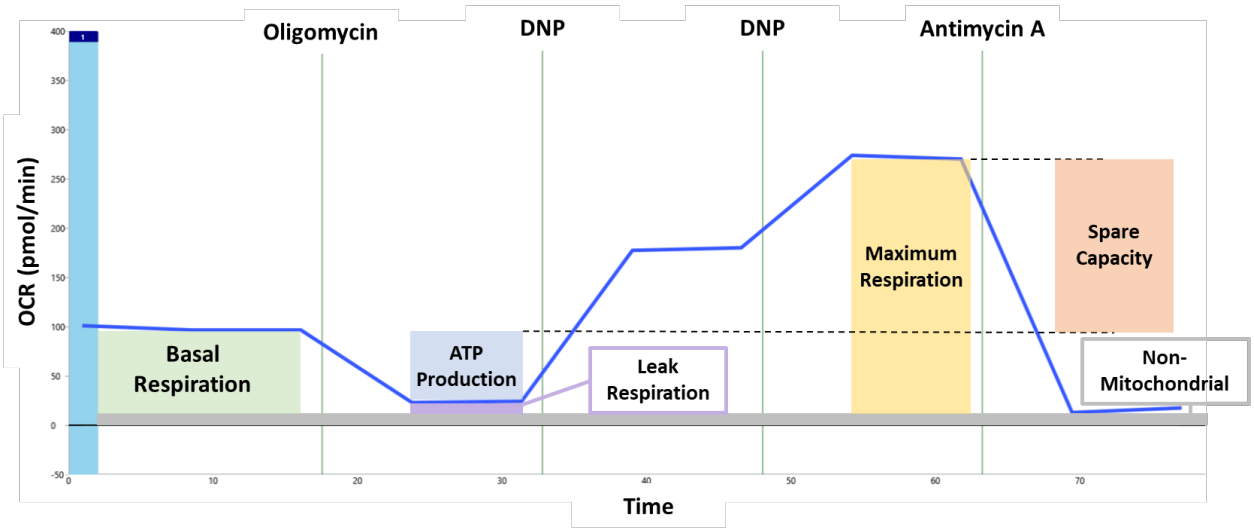

Figure S2: Description of cellular OCR metrics from a typical Seahorse run.

Table S2: Detailed protocol for Seahorse bioenergetics analysis. Detailed injection and measurement protocol for oxygen consumption and extracellular acidification measurements for platelets and PBMCs. Each Measurement cycle includes a mix step, a wait step, and ends with a measurement step for both OCR and ECAR. Drug concentrations represent final concentrations in each well after injection.

Action	Estimated time into run
Measurement 1	7:00
Measurement 2	14:00
Measurement 3	21:00
Injection 1 (2.5 µM Oligomycin)	22:00
Measurement 4	29:00
Measurement 5	36:00
Injection 2 (DNP: 30 µM for platelets, 120 µM for PBMCs)	37:00
Measurement 6	44:00
Measurement 7	51:00
Injection 3 (DNP: 30 µM for platelets, 40 µM for PBMCs)	52:00
Measurement 8	59:00
Measurement 9	66:00
Injection 4 (10 µM Antimycin A)	67:00
Measurement 10	74:00
Measurement 11	81:00

Supplemental Results

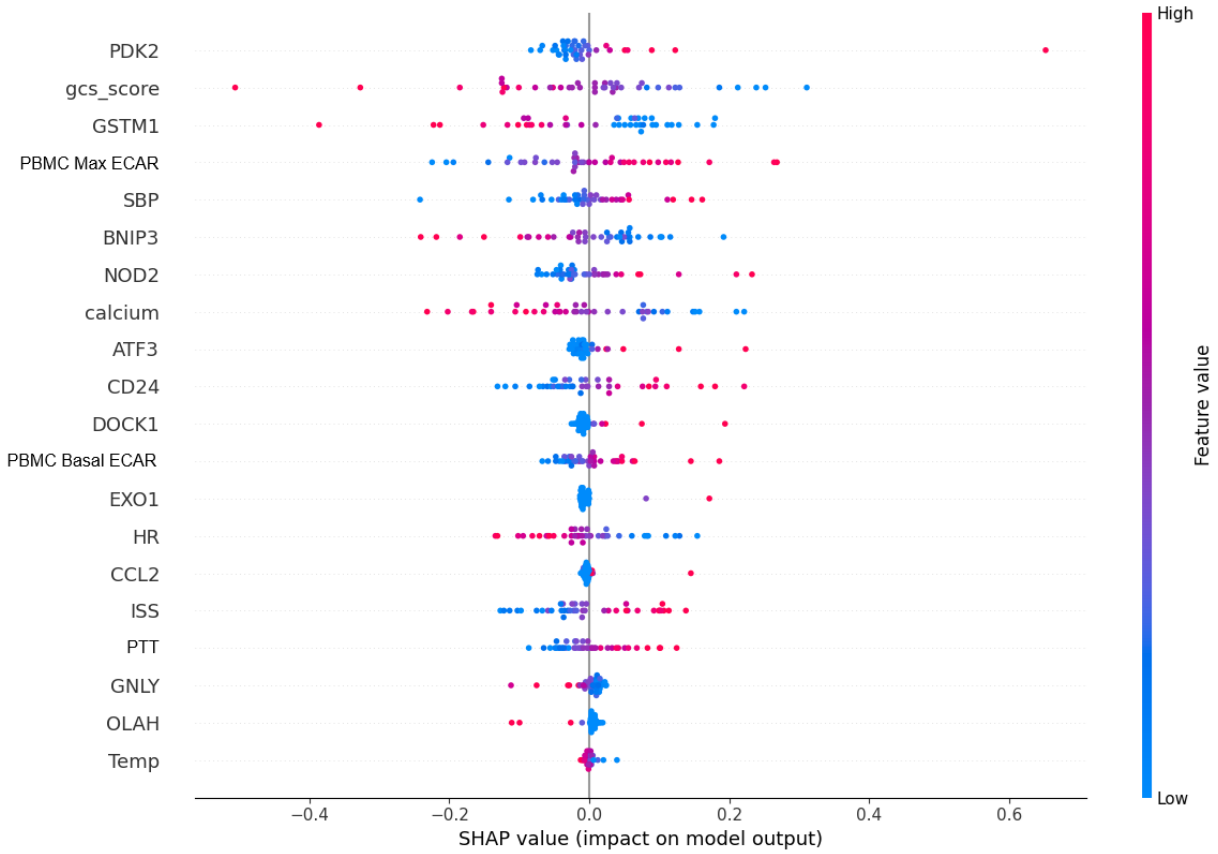

Figure S3: Variable importance ranking via SHAP values for LASSO model. The SHAP values are used to explain how much each variable contributes to the difference between the actual output and the expected output of a model for a given input data point. Each dot is a patient's value for a variable. Blue means its value is low and red is high. A dot located on the right side means the value of that variable increases the chance of developing SOF. A dot on the left side means the value of that variable decreases the chance of developing SOF.

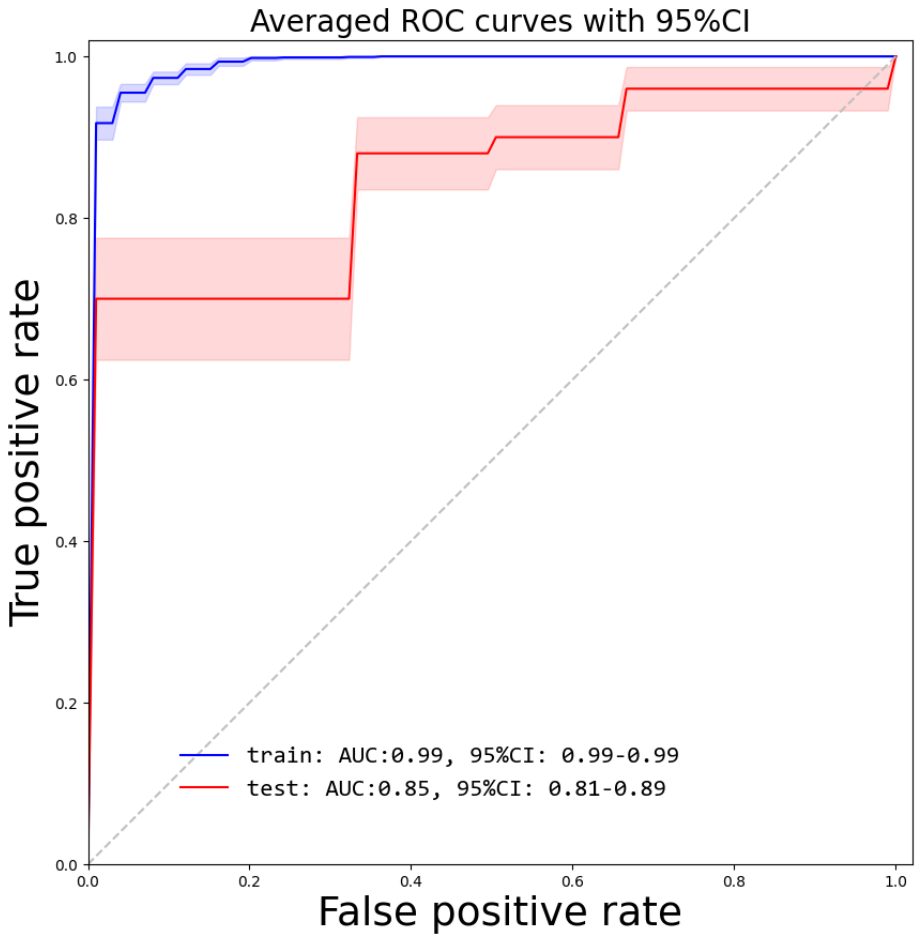

Figure S4: AUROCs of a LASSO regression without normalization. This is to evaluate the model's performance on unseen data. The area under the receiver operating characteristic curve is used as the performance metric. The blue curve is for training and the red curve is for testing. The results showed that the LASSO and XGB models performed similarly well (LASSO AUC 0.85, 95% CI 0.81-0.89, XGB AUC 0.82, 95% CI 0.78-0.86) on the testing dataset.

Conference Posters

University of Maryland School of Medicine Anesthesiology Department Research Retreat, Baltimore, MD, Oct 2022.

Genomics- and Bioenergetics-Based Personalized Treatment for Trauma Patients at Risk for Sepsis and Organ Failure

Apurva Borcar[1], Jennifer Klinedinst[2], Parisa Rangghran[3], Rosemary Kozar[4], Deborah Stein[4], Claire Fraser[5], and Gary Fiskum[3]

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Systemic, non-neurological complications such as sepsis and organ failure are common after severe traumatic brain injury (TBI). Sepsis occurs in 50-75% of patients with severe TBI and can lead to multiple organ system failure, but the search for early-detection biomarkers for sepsis is ongoing. This active clinical study examines the correlations between blood cell gene expression and bioenergetics with the clinical observations and long-term outcomes of severe TBI patients admitted to the R. Adams Cowley Shock Trauma Center in order to identify prognostic biomarkers for sepsis and organ failure. Our hypothesis states that gene expression and mitochondrial health of circulating blood cells sense stress in patients and may serve as biomarkers of human pathologies such as sepsis. Gene expression is investigated by whole blood RNA isolation and analysis via Nanostring nCounter® over the course of 7 days post-injury. Similarly, fresh blood is drawn over the first 7 days for isolation and bioenergetic examination of PBMCs. These data sets will be compared to clinical data with the purpose of identifying novel biomarkers as early diagnostic tools and improving outcomes of patients with severe TBI.

[This protocol was reviewed and approved by the University of Maryland, Baltimore, Institutional Review Board (IRB) and the US Air Force IRB. Air Force Co-Op number FA8650-19-2-6H19.]

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Introduction

Systemic, non-neurological complications such as sepsis and organ failure are common after severe traumatic brain injury (TBI). Sepsis occurs in 50-75% of patients with severe TBI and can lead to multiple organ system failure, but the search for early-detection biomarkers for sepsis is ongoing. This clinical study examines the correlations between blood cell gene expression and bioenergetics with the clinical observations and long-term outcomes of severe TBI patients admitted to the R. Adams Cowley Shock Trauma Center in order to identify prognostic biomarkers for sepsis and organ failure.

Our hypothesis states that gene expression and mitochondrial health of circulating blood cells can reveal stress in patients and may serve as biomarkers of human pathologies such as sepsis. Gene expression is investigated by whole blood RNA isolation and analysis via Nanostring nCounter® over the course of 7 days post-injury. Similarly, fresh blood is drawn over the first 7 days for isolation and bioenergetic examination of peripheral blood mononuclear cells (PBMCs). These data sets will be compared to clinical data with the purpose of identifying novel biomarkers as early diagnostic tools and improving outcomes of patients with severe TBI.

Methodology

Potential enrollees admitted to the R. Adams Shock Trauma Center are radiologically screened for TBI.

Inclusion criteria

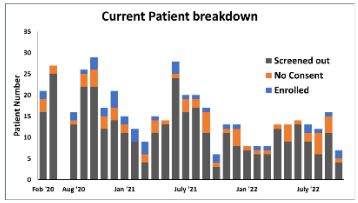
- ✓ Age at least 18 years
- ✓ Presence of intracranial traumatic pathology on head CT
- ✓ Clinically indicated to be admitted to the University of Maryland Shock Trauma MTICU or NTICU
- ✓ Can speak and understand English

Exclusion criteria

- ❖ Hemodynamically unstable
- ❖ Transfer from another hospital more than 24 h after head injury
- ❖ Determination of non-survivability on admission by neurosurgical team
- ❖ Weight < 110 lbs
- ❖ Currently incarcerated (i.e., prisoners)
- ❖ Pregnant

- Once a patient is enrolled:
- Blood is drawn into Tempus tubes for RNA stabilization on Days 0 (upon admission), 1, 2, 4, and 7
- Blood is drawn into EDTA tubes for bioenergetic analysis on Days 1, 2, 4, and 7
- Clinical data is collected for the patient throughout treatment for up to 30 days.

Enrollment



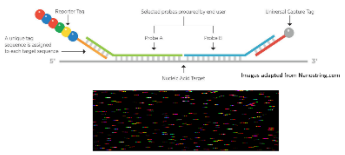
Current enrollees: 41 patients (out of 50)

Contributors to low numbers:

1. COVID (shutdown between 3/18/20 and 8/18/20) and aftermath
2. Very low TBI patient number
3. No LAR consent within 48 hr

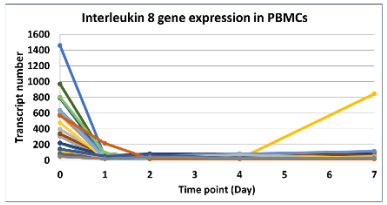
Transcriptomics

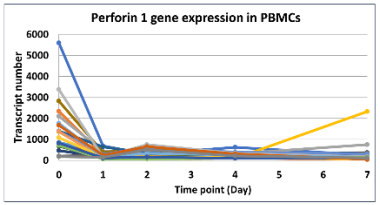
Blood collected in Tempus RNA stabilization tubes is processed by the UMB Center for Innovative Biomedical Resources (CIBR) and run on the Nanostring nCounter® platform by the UMB Institute for Genome Sciences (IGS). Nanostring uses unique color-coded tags to identify and count nucleic acid transcripts.



Our custom 800-gene panel includes transcripts involved in: inflammation, immunity, oxidative stress, cell typing, apoptosis, and hemostasis.

Although comparative analysis to sepsis/organ failure is forthcoming, preliminary data has yielded unexpected results:





The data above represents 21 patients. All data sets were normalized together using positive controls in each sample. Each line represents a separate patient over the 7-day period of sample collection and the colors are consistent with the patients for both genes. Expression of both Interleukin-8 and Perforin 1 greatly decrease after admission (Day 0). Only one patient showed a significant increase in the expression of both genes at day 7. Interleukin-8 (IL-8) is an inflammatory cytokine and a major mediator of the inflammatory response, and it would be expected to increase. Perforin 1 (PRF1) plays a role in the immune response by creating membrane pores for purposes of cytolysis (degradation of target cells).

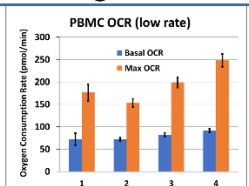
Bioenergetics

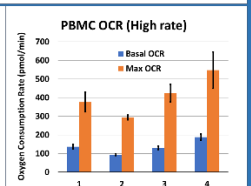
PBMCs are isolated via immunomagnetic separation using the StemcellTM EasySepTM Direct Human PBMC Isolation Kit within 6 hr of blood draw and plated for respirometric analysis utilizing an Agilent Seahorse XFe24 Analyzer.

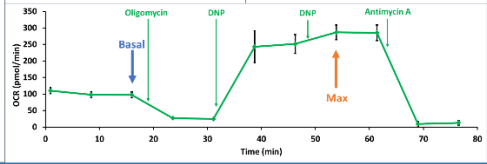
The mito-stress test protocol gives a view of the overall health and efficiency of the mitochondria in PBMCs.

Top figures: Oxygen consumption rates (OCRs) for 2 enrolled patients that show the range of measurements between patients. (n=5-10 for each data point)

Bottom figure: Representative image of an oxygen consumption trace by isolated PBMCs







Clinical Data

Individual patient information

- Demographics
- Injury
- Social Behavior (smoking, drugs, alcohol)
- Past medical history
- AIS, ISS, SOFA scores
- Presence of sepsis
- Nature of organ failure
- Length of stay, death date (if applicable)

Clinical Metrics

- Daily vital signs (temperature, blood pressure, heart rate, SpO2)
- Daily pain scale and score
- Daily GCS (eye, verbal, motor)
- Daily ICP
- Medications
- Surgical procedures
- Imaging
- EKG and EEG
- Line insertions and IV fluids
- Feeding tubes
- Intubation
- Blood transfusions
- Infections
- Urinalysis
- Blood work (toxicology, chemistry panel, blood gas, coagulation panel)

Future Studies

Upon completion of enrollment, bioinformatic analysis will compare gene expression, bioenergetic, and clinical data to indicate potential biomarkers for the onset of sepsis. Banked samples may be used for further analysis such as proteomics or miRNA profiling.

Acknowledgements

The authors would like to thank the nursing and Shock Trauma and Anesthesiology Research (STAR) staff at the R. Adams Cowley Shock Trauma Center for their invaluable help in enrolling patients and collecting samples. We would also like to thank the staff at CIBR and IGS for their continued help in processing samples.

Approvals and Disclaimers

The protocol was reviewed and approved by the University of Maryland, Baltimore, Institutional Review Board (IRB) and the United States Air Force IRB, Air Force Co-Op number FA8650-19-2-6H19.

The views expressed are those of the authors and do not reflect the official guidance or position of the United States Government, the Department of Defense, or of the United States Air Force.

University of Maryland School of Medicine Precision Medicine and Learning Health Systems Research Symposium, Baltimore, MD, 2023

Genomics- and Bioenergetics-Based Personalized Treatment for Trauma Patients at Risk for Sepsis and Organ Failure

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Systemic, non-neurological complications such as sepsis and organ failure are common after severe traumatic brain injury (TBI). Sepsis occurs in 50-75% of patients with severe TBI and can lead to multiple organ system failure, but the search for early-detection biomarkers for sepsis is ongoing. This clinical study examines the correlations between blood cell gene expression and bioenergetics with the clinical observations and long-term outcomes of severe TBI patients admitted to the R. Adams Cowley Shock Trauma Center in order to identify prognostic biomarkers for sepsis and organ failure. Our hypothesis states that gene expression and mitochondrial health of circulating blood cells sense stress in patients and may serve as biomarkers of human pathologies such as sepsis. Gene expression is investigated by whole blood RNA isolation and analysis via Nanostring nCounter® over the course of 7 days post-injury. Similarly, fresh blood is drawn over the first 7 days for isolation and bioenergetic examination of PBMCs. Through the use of machine learning techniques, these data sets will be compared to clinical data with the purpose of identifying novel biomarkers as early diagnostic tools and improving outcomes of patients with severe TBI.

[This protocol was reviewed and approved by the University of Maryland, Baltimore, Institutional Review Board (IRB) and the US Air Force IRB. Air Force Co-Op number FA8650-19-2-6H19.]

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UNIVERSITY of MARYLAND SCHOOL OF MEDICINE

Introduction

Systemic, non-neurological complications such as sepsis and organ failure are common after severe traumatic brain injury (TBI). Sepsis occurs in 50-75% of patients with severe TBI and can lead to multiple organ system failure, but the search for early-detection biomarkers for sepsis is ongoing. This clinical study examines the correlations between blood cell gene expression and bioenergetics with the clinical observations and long-term outcomes of severe TBI patients admitted to the R. Adams Cowley Shock Trauma Center in order to identify prognostic biomarkers for sepsis and organ failure.

Our hypothesis states that gene expression and mitochondrial health of circulating blood cells can reveal stress in patients and may serve as biomarkers of human pathologies such as sepsis. Gene expression is investigated by whole blood RNA isolation and analysis via Nanostring nCounter® over the course of 7 days post-injury. Similarly, fresh blood is drawn over the first 7 days for isolation and bioenergetic examination of peripheral blood mononuclear cells (PBMCs). These data sets will be compared to clinical data with the purpose of identifying novel biomarkers as early diagnostic tools and improving outcomes of patients with severe TBI.

Methodology

Potential enrollees admitted to the R. Adams Shock Trauma Center are radiologically screened for TBI.

Inclusion criteria

- ✓ Age at least 18 years
- ✓ Presence of intracranial traumatic pathology on head CT
- ✓ Clinically indicated to be admitted to the University of Maryland Shock Trauma MTICU or NTICU
- ✓ Can speak and understand English

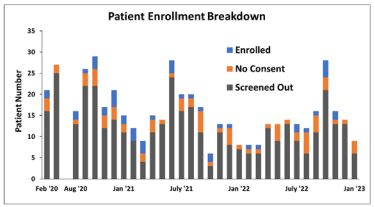
Exclusion criteria

- ❖ Hemodynamically unstable
- ❖ Transfer from another hospital more than 24 h after head injury
- ❖ Determination of non-survivability on admission by neurosurgical team
- ❖ Weight < 110 lbs
- ❖ Currently incarcerated (i.e., prisoners)
- ❖ Pregnant

After a potential enrollee is determined to be TBI positive:

- Blood is drawn into Tempus tubes for RNA stabilization on Days 0 (upon admission), 1, 2, 4, and 7
- Blood is drawn into EDTA tubes for bioenergetic analysis on Days 1, 2, 4, and 7
- Clinical data is collected for the patient throughout treatment for up to 30 days.

Enrollment



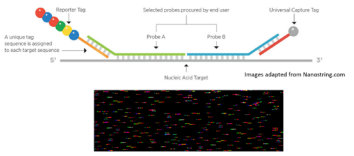
45 Total Enrollees

Contributors to low enrollment numbers:

1. COVID (shutdown between 3/18/20 and 8/18/20) and aftermath
2. Very low TBI patient number
3. No LAR consent within 48 hr
4. Supply chain issues for necessary materials

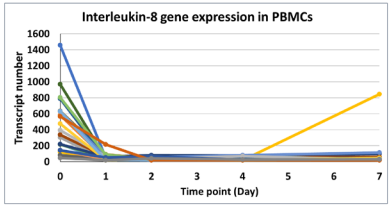
Transcriptomics

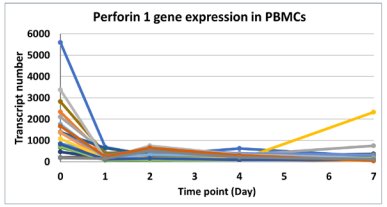
Blood collected in Tempus RNA stabilization tubes is processed by the UMB Center for Innovative Biomedical Resources (CIBR) and run on the Nanostring nCounter® platform by the UMB Institute for Genome Sciences (IGS). Nanostring uses unique color-coded tags to identify and count nucleic acid transcripts.



Our custom 800-gene panel includes transcripts involved in: inflammation, immunity, oxidative stress, cell typing, apoptosis, and hemostasis.

Although comparative analysis to sepsis/organ failure is forthcoming, preliminary data has yielded unexpected results:





The data above represents 21 patients. All data sets were normalized together using positive controls in each sample. Each line represents a separate patient over the 7-day period of sample collection and the colors are consistent with the patients for both genes. Expression of both Interleukin-8 and Perforin 1 greatly decrease after admission (Day 0). Only one patient showed a significant increase in the expression of both genes at day 7. Interleukin-8 (IL-8) is an inflammatory cytokine and a major mediator of the inflammatory response, and it would be expected to increase. Perforin 1 (PRF1) plays a role in the immune response by creating membrane pores for purposes of cytolysis (degradation of target cells).

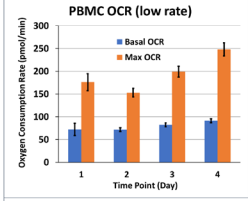
Bioenergetics

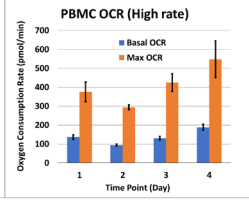
PBMCs are isolated via immunomagnetic separation using the Stemcell™ EasySep™ Direct Human PBMC Isolation Kit within 6 hr of blood draw and plated for respirometric analysis utilizing an Agilent Seahorse XFe24 Analyzer.

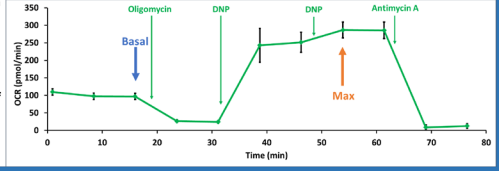
The mito-stress test protocol gives a view of the overall health and efficiency of the mitochondria in PBMCs.

Top figures: Oxygen consumption rates (OCRs) for 2 enrolled patients that show the range of measurements between patients. (n=5-10 for each data point)

Bottom figure: Representative image of an oxygen consumption trace by isolated PBMCs







Clinical Data

Individual patient information

- Demographics
- Injury
- Social Behavior (smoking, drugs, alcohol)
- Past medical history
- AIS, ISS, SOFA scores
- Presence of sepsis
- Nature of organ failure
- Length of stay, death date (if applicable)

Clinical Metrics

- Daily vital signs (temperature, blood pressure, heart rate, SpO2)
- Daily pain scale and score
- Daily GCS (eye, verbal, motor)
- Daily ICP
- Medications
- Surgical procedures
- Imaging
- EKG and EEG
- Line insertions and IV fluids
- Feeding tubes
- Intubation
- Blood transfusions
- Infections
- Urinalysis
- Blood work (toxicology, chemistry panel, blood gas, coagulation panel)

Analysis and Additional Studies

We have begun analysis of the bioenergetics and transcriptomics data for correlations to sepsis/organ failure as determined by clinical observations. We are also using banked plasma samples to measure the levels of cytokines and catecholamines as other potential diagnostic biomarkers.

Acknowledgements

The authors would like to thank the nursing and Shock Trauma and Anesthesiology Research (STAR) staff at the R. Adams Cowley Shock Trauma Center for their invaluable help in enrolling patients and collecting samples. We would also like to thank the staff at CIBR and IGS for their continued help in processing samples.

Approvals and Disclaimers

The protocol was reviewed and approved by the University of Maryland, Baltimore, Institutional Review Board (IRB) and the United States Air Force IRB, Air Force Co-Op number FA8650-19-2-6H19.

The views expressed are those of the authors and do not reflect the official guidance or position of the United States Government, the Department of Defense, or of the United States Air Force.

LIST OF ACRONYMS
(For gene information, see **Table S1**)

711 HPW/RHBAM	Air Force Research Laboratory, 711th Human Performance Wing, Airman Systems Directorate, Airman Biosciences Division, Product Development Branch, Enroute Care Section
%	percent
°C	Degree Celsius
mM	millimolar
ng	nanogram
pmol/min	picomoles per minute
mpH/min	milliPH per minute
pH	potential hydrogen
pg/ml	picograms per milliliter
ng/ml	nanograms per minute
µg	microgram
µl	microliter
µM	micromolar
AIS	Abbreviated injury score
ATP	Adenosine triphosphate
AUC	Area under the curve
AUROC	Area under the receiver operating characteristics curve
BP	blood pressure
CCL5	Chemokine C-C motif ligand 5
CI	Confidence interval
CT	computerized tomography

cDNA	circular DNA
diH_2O	deionized water
DMEM	Dulbecco's Modified Eagle's Medium
DNA	deoxyribonucleic acid
DNP	2,4-dinitrophenol
DPI	Days post injury
ECAR	extracellular acidification rate
EDTA	Ethylenediaminetetraacetic Acid
ELISA	Enzyme-linked immunosorbent assay
XGB	Extreme gradient boosted
FBS	Fetal bovine serum
GCS	Glasgow Coma Scale
g-force	gravitational force
FDR	false discovery rate
HIPAA	Health Insurance Portability and Accountability Act
HR	Heart rate
ICU	intensive care unit
IFN-ɣ	interferon-ɣ
IRB	institutional review board
IL6	interleukin 6
ISS	Injury severity score
LAR	legally authorized representative
LASSO	least absolute shrinkage and selection operator

LOOCV	leave one out cross-validation
MIP1β	macrophage inflammatory protein 1β
MCP-1	monocyte chemoattractant protein-1
min	minutes
miRNA	micro RNA
mRNA	messenger RNA
MRMR	Maximum relevance minimum redundance
MTICU	Medical intensive care unit
NaCl	Sodium Chloride
NTICU	Neurotrauma intensive care unit
NonSOF	never diagnosed with SOF
OCR	Oxygen consumption rate
PBMCs	Peripheral blood mononuclear cells
PBS	phosphate buffered saline
ProgSOF	prognostic for sepsis and/or organ failure
PTT	Partial thromboplastin time
qRT-PCR	real-time polymerase chain reaction
RANTES	regulated on activation, normal T cell expressed and secreted
ROC	receiver operating characteristic curve
RNA	ribonucleic acid
RMS	Research Management System
SBP	systolic blood pressure
SHAP	Shapley additive explanations

SI	Shock index
SOF	sepsis and/or organ failure
SpO2	Peripheral oxygen blood saturation
STC	Shock Trauma Center
STAR	Shock, Trauma, and Anesthesiology Research Center
SOFA	sequential organ failure assessment score
TBI	traumatic brain injury
TNF alpha	tumor necrosis factor alpha
TRU	trauma resuscitation unit
UMSOM	University of Maryland School of Medicine
XGB	Extreme gradient boosting
US	United States