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Early alterations in platelet mitochondrial function are associated with survival and organ failure in patients with septic shock

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Abstract

Introduction—To determine if changes platelet mitochondrial function in patients with sepsis are present early following presentation, and the association of these changes with clinical outcomes and systemic metabolic function.

Materials and Methods—Prospective observational cohort study of a convenience sample of patients with severe sepsis. Mitochondrial function of intact, non-permeabilized platelets suspended in their own plasma was estimated using high resolution respirometry. Unstimulated basal respiration, oligomycin-induced state 4 (state 4o) and maximal respiratory rate following serial titrations of FCCP were measured. Organ failure was estimated using SOFA score, and patients were followed until 28 days to determine survival. Lactate levels were measured in all patients, and a subset of patients had lactate:pyruvate ratios measured.

Results—28 patients were enrolled, 21 of whom survived. Initial SOFA score and lactate levels were 8.5 (IQR 6, 10) and 2.3 (IQR 1.2, 3.5) respectively, while the median L/P ratio was 23.4 (IQR 15.2, 38). Basal and maximal respiratory rates were significantly higher among non-survivors compared to survivors (p=0.02 and 0.04), while oligomycin-induced state 4 respiration was not statistically different between groups (p = 0.15). We found a significant association between maximal respiration and organ failure (p = 0.03), and both basal and maximal rates with initial lactate level (p = 0.04, 0.02), but not with lactate:pyruvate ratio.

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This work was performed at the University of Mississippi Medical Center.

Conclusions—Differences in platelet mitochondrial function between survivors and non-survivors are present very early in the hospital course and are associated with organ failure and lactate.

Keywords

sepsis; metabolism; oxygen consumption; prognosis; biomarker; lactate; pyruvate; organ failure

Introduction

In the setting of an aging patient population with an increasing comorbidity burden, the incidence of severe sepsis and septic shock are increasing.(1) Meanwhile, mortality remains high for the two conditions, at greater than 20%(1) and 40%,(2) respectively. Decades of preclinical and clinical research have yielded significant improvement understanding of the pathophysiology of sepsis, particularly in regards to the critical role played by the inflammatory cascade. Unfortunately, clinical trials regarding specific pharmacologic therapies for the treatment of sepsis have had a checkered history and have not resulted in bringing novel, efficacious drugs into routine clinical practice. Given this history, investigations into relatively under investigated components of the pathophysiologic cascade is critical for the development of novel therapeutics.

Mitochondrial dysfunction is increasingly recognized as playing a critical role in the development and persistence of organ failure in sepsis.(3;4) This hypothesis may help explain many key features of death from sepsis including the notable lack of widespread apoptosis or necrosis within tissues, which suggests against hypoxia as the primary driver of organ failure. While animal models of sepsis exhibit mitochondrial and subsequent organ dysfunction in widespread vital organs including the heart(5) and kidneys,(6) access to the tissues necessary to study mitochondria have limited translation of these findings to humans. To date, human studies support the existence of mitochondrial dysfunction in humans with severe sepsis in a number of accessible cell lines, including leukocytes,(7) platelets,(8) and skeletal muscle.(9)

However, several questions remain under investigated and serve as the motivation for the present study. First, the timing of onset of altered mitochondrial respiration in sepsis remains unclear, which has implications for the design of interventional trials. Second, the association of platelet mitochondrial function and evidence of vital organ dysfunction or systemic perturbations in metabolism are lacking. To our knowledge, only a single small study by a single group has evaluated the prognostic potential of platelet mitochondrial alternations in sepsis.(8) In this study, we present a prospective cohort study of platelet mitochondrial function in patients with septic shock enrolled shortly after the initial diagnosis and resuscitation. The goal of this study was to test the hypothesis that alternations in early platelet mitochondrial function are associated with patient outcomes including survival and organ failure, and changes in systemic evidence of metabolism.

Materials and Methods

Study Design

This is was prospective observational study of a convenience sample of patients with severe sepsis enrolled at a single academic, tertiary care hospital from September 2013-November 2014. Patients were eligible for inclusion if they had 1) a suspected or confirmed infection; 2) any two of four criteria of systemic inflammatory response as defined by the 2001 ACCP/SCCM Consensus Conference Committee(10) and 3) a mean arterial pressure of ≤65 mmHg OR SBP <90 mmHg after 2L fluids or initiation of vasopressors. Exclusion criteria were 1) any primary diagnosis other than sepsis; 2) an established Do Not Resuscitate status or advanced directives restricting aggressive care or treating physician deems aggressive care unsuitable; 3) cardiopulmonary resuscitation (chest compression or defibrillation) prior to enrollment; or 4) if the principal investigator was not available to conduct high resolution respirometry. All patients or their surrogates provided written, informed consent and the study was approved by the local institutional review board.

Platelet isolation

Following consent, ~10 mL whole blood was drawn into EDTA vaccuntainers and gently inverted 6–8 times. The blood was taken immediately to the laboratory where it was centrifuged within 15 minutes at 300g × 15 minutes at room temperature. The resulting platelet rich plasma was transferred to a separate tube and centrifuge for an additional 5 minutes at 4,500g at room temperature, which yielded a platelet pellet and nearly cell-free plasma.

Platelet mitochondrial measurements

We adapted previously published methods for the measurement of platelet mitochondrial function in intact platelets.(8) All measurements were performed by a single operator blinded to the clinical status of the patient. Due to a smaller volume of blood than utilized in previous reports, and findings that suggests platelet mitochondrial abnormalities in sepsis are more pronounced in and mediated by a yet unknown factor in the serum,(7;8) all experiments were completed in the patient's plasma rather than buffered media. As a result, cells could not be permeabilized with digitonin. Therefore, the resultant oxygen consumption measurements do not represent classical mitochondrial states, though are consistent with prior measurements reported in the literature(8) and consistent with recommendations for the assessment of mitochondrial function in whole cells by experts in the field.(11)

Oxygen consumption was measured using a high resolution respirometer (Oxygraph O2k, Innsbruck, Austria). The instrument was calibrated following manufacturer instructions using 2 mL of plasma. Simultaneously, the platelet pellet was gently resuspended in 100–200 μL plasma, and the platelet count of the resulting ultra-platelet rich plasma was measured using an automated platelet counter (Cellometer AutoM10; Lawrence, MA). The necessary volume required to yield a final cell count of ~200×10^6 platelets / mL in the Oxygraph chamber was calculated and added to the chamber. This platelet count was chosen based on published data suggesting this concentration yields superior results.(12) The final

platelet concentration in the chamber was measured and entered into the manufacturer provided software (DatLab 5.2, Innsbruck, Austria) which normalized the results to cell count following completion of the experiment and the oxygen solubility constant in serum was estimated at 0.89. The chamber was closed, and following equilibration, the basal oxygen consumption rate of unstimulated platelets in plasma was estimated.

All chemicals for platelet mitochondrial experiments were purchased from Sigma-Aldrich (St. Louis, MO). Oligomycin was added to the chamber (final concentration in chamber 5 μM), effectively blocking ATP synthase activity to measure oligomycin induced state 4 (state 4o) respiration. Serial additions of carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone (FCCP) (1 μL; 20 mM) were added until a maximal respiration rate was obtained. Of note, this concentration is significantly higher (~20 fold) than the concentration required when performing similar experiments in buffer, but is consistent with work by other groups.(13) FCCP additions were continued until 3 consecutive additions failed to increase the respiration rate. Finally, rotenone and antimycin A (final concentrations 0.6 μM and 1.8 mM, respectively) were added consecutively to arrive at a final, residual oxygen consumption rate that is independent of electron transfer chain activity. Given the fact these measurements were performed in serum rather than buffer, this rate includes not only extra mitochondrial platelet oxygen consumption, but the oxygen consumption of the serum, as well. This residual rate was subtracted from the basal, state 4o, and maximal respiration rates.(11) The cellular respiratory control ratio (RCR) was calculated by dividing the corrected maximal respiration rate by the state 4o rate. The cellular RCR similarly differs from isolated mitochondrial RCR as maximal FCCP-induced and oligomycin-induced state 4 respiratory rates are not identical to classical state 3 and state 4 rates.(11)

Clinical measurements

Patient demographics, clinical characteristics, and outcomes were recorded by clinical research coordinators blinded to the results of the study measurements. Severity of illness at enrollment was estimated by the measurement of the Sequential Organ Failure Assessment (SOFA) score. All patients had lactate measurement performed as part of routine clinical care prior to inclusion in the study. Patients were followed to 28 days to determine survival.

Lactate : pyruvate (L:P) ratio and lactate clearance

In a subgroup of patients, additional samples were collected and processed at enrollment and 6 (± 1) hours later according to protocols provided by LabCorp Clinical Trials, (Cincinnati, OH), who performed the additional studies. Lactate:pyruvate (L:P) ratio was determined by dividing lactate by pyruvate. Relative lactate clearance was determined by calculating the difference between initial and delayed lactate and dividing by the initial lactate.

Outcomes and data analysis

The primary outcome of the study was the association between SOFA score and corrected platelet mitochondrial oxygen consumption at enrollment. Secondary outcomes included difference in platelet measurements between survivors and non-survivors, and the association between these same measurements and initial lactate, L:P ratio, and 6-hour relative lactate clearance. Simple descriptive statistics were used to summarize patient data.

Associations were tested by using the outcome of interest as the dependent variable and platelet respiratory rates as the independent variable in a linear regression model. Differences in mitochondrial measurements between survivors and non-survivors were tested using Student's t-tests. We tested for differences in each measure of platelet mitochondrial function between patients with literature based, *a priori* cutoffs. In the case of L:P ratio, values of greater than or ≤ 18 have been suggested to be a useful marker to distinguish hyperlactatemia from ischemic versus other causes.(14) Differences in relative lactate clearance of ≥ or <10% were chosen based on data supporting the poor prognostic value of impaired lactate clearance,(15;16) in order to test the hypothesis that lactate non-clearance despite hemodynamic resuscitation may be a marker for non-ischemic sources of lactate. Finally, we conducted a post-hoc analysis to compare platelet measurements among patients who were and were not receiving vasopressors. Commercially available software (STATA 10.1, College Station, TX and StatsDirect 2.8.0, Cheshire, England) were utilized for data analysis. Figures were generated using Microsoft Excel 2013 (Redmond, WA). All tests were two-sided and $p < 0.05$ were considered significant.

Results

We enrolled 28 patients in the study, 21 of whom survived to 28-days. At enrollment, median SOFA score of enrolled patients was 8.5 [Interquartile range (IQR) 6, 10], and initial lactate was 2.3 mmol/L (IQR 1.2, 3.5). Patient demographics and clinical characteristics of the entire cohort are summarized in Table 1. Patients received a median of 3.1 L (IQR 1.8, 3.8) intravenous fluid prior to enrollment, and 71% of patients were receiving vasopressors, suggesting patients were aggressively resuscitated. Non-survivors were more likely to have a history of congestive heart failure, but otherwise baseline demographics did not differ significantly between groups. Lactate:pyruvate (L:P) measurements were performed on a convenience sample of 13 patients at 0 and 6 hours. Median L:P ratio was 23.4 (IQR 15.2, 38). 9 (69%) patients met our *a priori* L:P ratio cutoff of >18, while 4 (31%) had a L:P ratio ≤ 18. Median lactate clearance was 7% (IQR −10%, 30%), while 7 (54%) cleared lactate at least 10% at 6 hours.

Platelet mitochondrial measurements were performed a median of 4.4 hours after hospital presentation. The median final platelet count in the O2k chamber following processing was $188 * 10^6$ platelets / mL (IQR 166, 224). On average, 4–5 FCCP additions were required to reach the maximal respiratory rate. Consistent with previous literature regarding human platelets, we found no further inhibition of respiration after addition of antimycin A following the addition of rotenone, suggesting almost complete use of complex I for electron transport in platelets.

We found a significant association between the primary outcome of SOFA score and maximal platelet respiration ($p = 0.04$), but not basal ($p = 0.08$) or state 4o ($p = 0.80$) oxygen consumption rates. However, the overall association was fairly weak ($R^2 = 0.19$), and seemingly driven by only a few patients. Both basal and maximal respirations were significantly higher among non-survivors compared to survivors ($p = 0.04$ and 0.04). State 4o respiration was similarly higher in non-survivors, but did not reach statistical significance ($p = 0.15$). These data are illustrated in Figure 1. Median cellular RCR was 5.5 (IQR 4.4,

7.3), but demonstrated no significant association with initial SOFA score (p = 0.84). The relationship between cellular RCR and initial lactate approached statistical significant (R^2 = 0.11, p = 0.06).

In terms of one of our secondary outcomes, the basal and maximal respiration demonstrated a significant association with initial lactate (R^2 = 0.16 and 0.22; p = 0.04 and 0.02 respectively), while no indication of association with state 4o respiration was observed (p = 0.86). As a hypothesis generating study, we wished to examine the relationship of L:P ratios to platelet mitochondrial function, with the hypothesis that platelet mitochondrial function was representative of whole body mitochondrial function, which might be reflected in altered L:P ratios. When the data were examined continuously, we found no association between any of the platelet measures and L:P ratio (p = 0.51 to p = 0.80). Similarly, we found no difference in any of these measures when an *a priori* literature derived cutoff of 18 was used to separate patients (Figure 2). There was no association between platelet mitochondrial measurements and lactate clearance (p = 0.81 to 0.98), but non-significantly higher basal and maximal rates in patients without compared to with a relative lactate clearance of ≥10% (p = 0.10 to 0.08).

Following our observation that all of the platelet measurements were higher in non-survivors, we decided to investigate the hypothesis that increased respiration across all respiratory rates may be secondary to increased catecholamines from exogenous vasopressors. We conducted a post-hoc analysis of patients receiving vasopressors compared to those not requiring vasopressors. We found no significant differences in unstimulated (p = 0.08), State 4o (p = 0.96), or maximal (p = 0.20) platelet mitochondrial function between patients receiving or not receiving vasopressors, suggesting that exogenous catecholamines were not driving our observed differences.

Discussion

In this prospective observational study, we enrolled 28 patients with severe sepsis and conducted high resolution respirometry to estimate platelet mitochondrial function. This study represents an external validation of the prognostic value of platelet mitochondrial function among patients with severe sepsis. Additionally, this study is the earliest such study to date, with patients enrolled a median of just over 4 hours from hospital presentation, demonstrating that these changes are presents very shortly after hospital presentation, which has potential implications for the development of therapeutics in the future. To our knowledge, this is the first study that has demonstrated a significant association between mitochondrial function from peripheral blood cells and measures of severity of illness, specifically in this study SOFA score and lactate, though the strength of these associations are only fair in relationship to SOFA score (R^2 = 0.19) and lactate (R^2 = 0.16–0.22). In addition, our data are consistent with previous literature, demonstrating platelet oxygen consumption is increased in non-survivors compared to survivors in the earliest hours following hospital presentation. These data were measured at an earlier time point than previous studies, demonstrating such alterations are present very early in the hospital course. However, we found no association with our hypothesized systemic reflections of altered

mitochondrial function, namely lactate:pyruvate ratio or lactate clearance, though this hypothesis generating subgroup was relatively small.

The difference in platelet mitochondrial function is apparent between survivors and non-survivors shortly after hospital presentation. This has potential implications for future investigations regarding novel metabolically targeted interventions. Some data suggest altered mitochondrial respiration in sepsis is mediated by a yet uncharacterized factor in the serum.(7;8) However, it remains unclear if changes in mitochondrial function are due to increased aerobic capacity, direct damage to the mitochondria, or occur in response to decreased substrate supply (including oxygen and metabolic substrates) (17). If the last of these is true, it would suggest that interventions aimed at early restoration of perfusion is of the utmost importance in sepsis, which is consistent with and would support the current clinical paradigm.(18) However, two pieces of evidence suggest this paradigm may have a narrow time frame and range of additional effectiveness in clinical practice. First, this study demonstrates that changes in mitochondrial function are present soon after hospital presentation. Patients in this study were aggressively resuscitated with intravenous fluids and vasopressors, limiting the potential room for further gains by restoration of hemodynamics and improvements in oxygen delivery. Second, these changes in mitochondrial function were measured in platelets, which by virtue of their location in systemic circulation are unlikely to be exposed to significant degrees of hypoxia. These data support the hypothesis that a factor in the serum rather than antecedent ischemia are responsible for the changes observed. These data are further supported by work by Sjovall, (8) demonstrating more substantial alterations in platelet mitochondrial function when experiments are performed in serum as opposed to buffer. The observations of Belikova(7) et al likewise support this hypothesis, as their group demonstrated that changes in mitochondrial oxygen consumption can by induced in healthy leukocytes by exposing them to serum from patients with sepsis, while leukocytes from septic patients can have disease-induced abnormalities mitigated by incubating them in healthy serum.

Consistent with previous literature in intact human platelets, oxygen consumption is increased, not decreased, in non-survivors. While we only measured one particular time point, previous studies have demonstrated these increases become even more prominent as patients are followed for several days.(8) The most striking finding in both our study, as well as other human studies, is the increase in maximal respiration rate. These data seem to stand in contradiction to data from some animal models, which demonstrate impaired (rather than increased) function of mitochondria from vital organs in sepsis. Suggested locations of inhibition that may be amenable to therapeutic manipulation include the electron transport chain, pyruvate dehydrogenase, and carnitine-palmitoyl-transferase.(20) However, data regarding platelet mitochondrial function in animal models and vital organ mitochondrial function in humans with sepsis are relatively lacking, making comparisons difficult. It remains unclear if these differences are due to differences between vital organs and peripheral blood cells, differences in timing of measurements between models (where the onset of disease is well defined) and humans (where the onset of disease is unclear), or whether current animal models fail to adequately represent the human phenotype. One potential insight from recent animal models is the observation of an inverse-U relationship between mitochondrial oxygen consumption over time. The authors demonstrated that

mitochondrial function increases early following the onset of sepsis and develops later adaptation,(21) suggesting that the timing of measurements may be of critical importance. A recent study of complex IV (cytochrome *c* oxidase, the final component of the electron transport chain) activity in nearly 200 septic patients does seem consistent with these data, however.(19) In this study by Lorente, complex IV activity, normalized by mitochondrial content, was found to be decreased in sepsis compared to control patients, and in non-survivors compared to survivors. Resolving these potentially disparate observations, particularly in humans, remains an important area for future research in the field. One potential explanation to reconcile these results suggested by Lorente is that an increase in ATP demand leads to decreased $\Delta\psi_m$ secondary to increased proton movement, which would ultimately facilitate increased oxygen consumption, provided the respiratory complex levels are not impaired to the point so as to be rate-limiting.

As a corollary, future research might also focus on the specific pathophysiologic consequences of mitochondrial dysfunction within a certain cell line. Specifically in the case of platelets, both increased state 4o and maximal respiration might be observed during the transition to a highly activated phenotype referred to as coated platelets.(22) This pathway is dependent upon the mitochondrial permeability transition pore, and results in a highly procoagulant phenotype characterized by high-level prothrombinase complex expression that might be expected to increase blood viscosity and promote microvascular coagulation. Further investigation of this pathway and its relevance to sepsis remains another area for future investigation.

One of the goals of this study was to provide evidence that platelet mitochondrial function is representative of vital organ and “whole-body” mitochondrial function. Biopsies from vital organs are extremely difficult to obtain in this patient population, limiting advancements in the field, and raising the need for surrogate measures. Recent evidence from a small-animal model of hemorrhagic shock suggests against the hypothesis that peripheral blood cells accurately reflect the mitochondrial function of deeper, vital organs. In contrast to these findings, this is the first study to our knowledge to demonstrate a significant association between platelet mitochondrial function and both organ failure and initial lactate. However, it is possible and indeed likely that such an association merely reflects overall severity of illness, and we are aware of no data to date directly demonstrating an association of mitochondrial function from peripheral blood cells and mitochondrial function of vital organs. Until such data are available, we urge caution in the use of platelets as an accurate reflection of mitochondrial function in specific vital organs or the whole body of animals or patients with sepsis.

There are several important limitations to consider with our study. First, while this is the largest study of its kind to date of which we are aware, the sample size is still relatively small and a larger study may have detected differences that our study was unable to detect, particularly in regards to associations with lactate:pyruvate ratios and lactate clearance measurements. Second, the study was necessarily a convenience sample based on principal investigator availability. However, the risk of bias was minimized through the use of consecutive patient screening given standardized procedures, and the fact that the PI was blinded to the clinical status of the patient until completion of the study. Third, we chose to

conduct our measurements in the patient's serum rather than a buffer, based on data suggesting this may be the more important measurement in patients with sepsis. This limited our ability to carefully interrogate the electron transport chain due to problems with permeabilizing cells in serum, and forced us to use estimates of mitochondrial function such as whole cell basal, oligomycin-induced state 4, and maximally stimulated oxygen consumption. Similarly, the volume of blood drawn was minimal, and our platelet samples did not contain sufficient protein to perform comprehensive measurements of specific protein activities, such as pyruvate dehydrogenase or components of the electron transport chain. Thus, while our ability to determine the specific location and pathophysiology behind the observed alterations was limited, we believe the measurements chosen have as much or more clinical relevance as specific complex measurements. However, determination of the cause of these alterations will remain an important avenue of future investigation. Finally, based on our design, we cannot state with any certainty the cause or consequences of the altered platelet mitochondrial function, but rather present the data here as an associative biomarker rather than a causative factor in the development of organ failure or death of the patient.

Conclusion

Platelet mitochondrial function as estimated by intact, whole cell high resolution respirometry in the serum differs between survivors and non-survivors very early following hospital presentation, and is associated with both organ failure and serum lactate. Further investigations into the pathophysiologic causes and consequences of such alterations are indicated.

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Reference List

1. Gaieski D, Edwards J, Kallan M, Carr B. Benchmarking the incidence and mortality of severe sepsis in the United States. Crit Care Med. 2013; 41(5):1167–1174. [PubMed: 23442987]
2. Kaukonen KM, Bailey M, Suzuki S, Pilcher D, Bellomo R. Mortality Related to Severe Sepsis and Septic Shock Among Critically Ill Patients in Australia and New Zealand, 2000–2012. JAMA. 2014 ePub ahead of print.
3. Fink MP. Bench-to-bedside review: Cytopathic hypoxia. Critical Care (London). 2002; 6:491–499.
4. Garrabou G, Moren C, Lopez S, Tobias E, Cardellach F, Miro O, et al. The Effects of Sepsis on Mitochondria. J Infect Dis. 2012; 205:392–400. [PubMed: 22180620]
5. Piel DA, Gruber P, Weinheimer C, Courtois M, Robertson C, Coopersmith C, et al. Mitochondrial resuscitation with exogenous cytochrome c in the septic heart. Crit Care Med. 2007; 35(9):2120–2127. [PubMed: 17855825]
6. Patil NK, Parajuli N, MacMillan-Crow LA, Mayeux PR. Inactivation of renal mitochondrial respiratory complexes and manganese superoxide dismutase during sepsis: mitochondria-targeted

antioxidant mitigates injury. Am J Physiol Renal Physiol. 2014; 306(7):F734–F743. [PubMed: 24500690]

7. Belikova I, Lukaszewicz A, Faivre V, Damosiel C, Singer M, Payen D. Oxygen consumption of human peripheral blood mononuclear cells in severe human sepsis. Crit Care Med. 2007; 35(12): 2702–2708. [PubMed: 18074472]
8. Sjovall F, Morota S, Hansson M, Gnaiger E, Elmer E. Temporal increase of platelet mitochondrial respiration is negatively associated with clinical outcome in patients with sepsis. Crit Care. 2010; 14(R214):1–11.
9. Brearley D, Brand M, Hargreaves I, Heales S, Land S, Smolenski R, et al. Association between mitochondrial dysfunction and severity and outcome of septic shock. Lancet. 2002; 360:219–223. [PubMed: 12133657]
10. Levy MM, Fink MP, Marshall JC, et al. 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. Crit Care Med. 2003; 31(4):1250–1256. [PubMed: 12682500]
11. Brand M, Nichols DG. Assessing mitochondrial dysfunction in cells. Biochem J. 2011; 435:297–312. [PubMed: 21726199]
12. Sjovall F, Ehinger JKH, Marelsson SE, Morota S, Frostner EA, Uchino H, et al. Mitochondrial respiration in human viable platelets - Methodology and influence of gender, age, and storage. Mitochondrion. 2013; 13:7–14. [PubMed: 23164798]
13. Sjövall, Fredrik. Personal Communication. 2013. p. 8-25.
14. Rimachi R, Bruzzi de Carvahlo F, Orellano-Jimenez C, Cotton F. Lactate/pyruvate ratio as a marker of tissue hypoxia. Anaesth Intensive Care. 2012; 40(3):427–432. [PubMed: 22577907]
15. Nguyen HB, Rivers EP, Knoblich BP, et al. Early lactate clearance is associated with improved outcome in severe sepsis and septic shock. Crit Care Med. 2004; 32(8):1637–1642. [PubMed: 15286537]
16. Puskarich M, Trzeciak S, Shapiro N, Albers AB, Heffner AC, Kline JA, et al. Whole Blood Lactate Kinetics in Patients Undergoing Quantitative Resuscitation for Severe Sepsis and Septic Shock. Chest. 2013; 143(6):1548–1553. [PubMed: 23740148]
17. Balestra G, Legrand M, Ince C. Microcirculation and mitochondria in sepsis: getting out of breath. Curr Opin Anes. 2009; 22:184–190.
18. Rivers E, Nguyen B, Havstad S, et al. Early goal-directed therapy in the treatment of severe sepsis and septic shock. N Engl J Med. 2001; 345(19):1368–1377. [PubMed: 11794169]
19. Lorente L, Martin MM, Lopez-Gallardo E, et al. Decrease of oxidative phosphorylation system function in severe septic patients. J Crit Care. 2015
20. Dare AJ, Phillips ARJ, Hickey ARJ, Mittal A, Loveday B, Thompson N, et al. A systematic review of experimental treatments for mitochondrial dysfunction in sepsis and multiple organ dysfunction syndrome. Free Radical Biology and Medicine. 2009; 47:1517–1525. [PubMed: 19715753]
21. Liu TF, Vachharajani V, Millet P, et al. Sequential actions of SIRT1-RELB-SIRT3 coordinate nuclear-mitochondrial communication during immunometabolic adaptation to acute inflammation and sepsis. J Biol Chem. 2015; 290(1):396–408. [PubMed: 25404738]
22. Remenyi G, Szasz R, Friese P, Dale GL. Role of Mitochondrial Permeability Transition Pore in Coated-Platelet Formation. Arterioscler Thromb Vasc Biol. 2004; 25:467–471. [PubMed: 15591217]

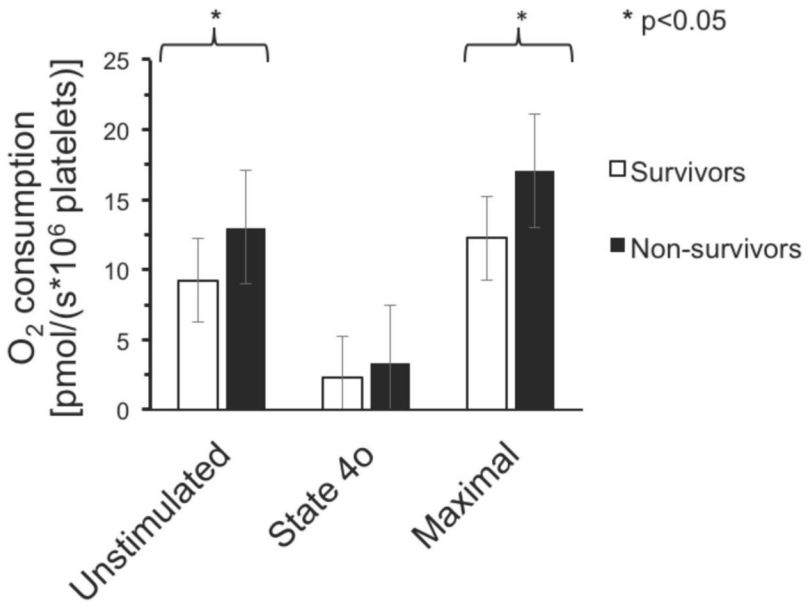

Figure 1.

Difference in platelet mitochondrial respiration rates between survivors and non-survivors. Error bars represent the standard error of the mean.

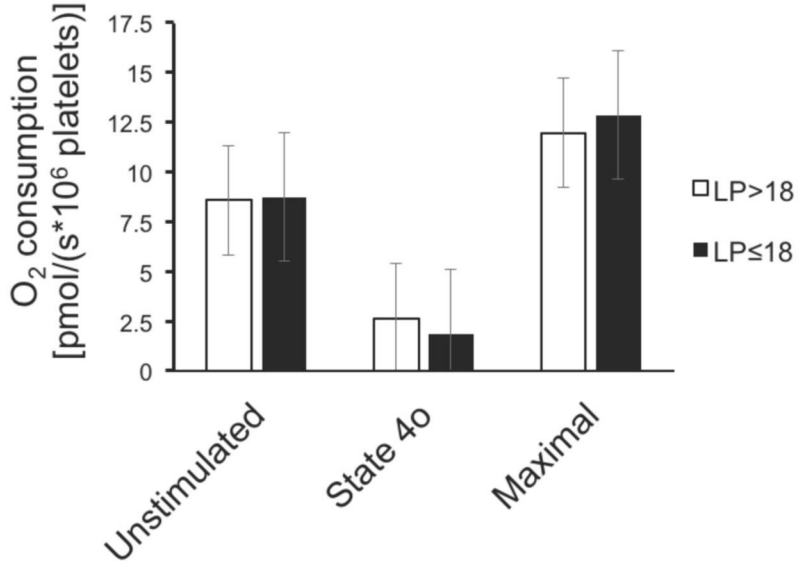

Figure 2.

Difference in platelet mitochondrial respiration rates between patients with a lactate:pyruvate ratio > or ≤ 18. Error bars represent the standard error of the mean. Of note, these data represent a convenience sample of only 13/28 patients.

Table 1

Patient demographics and clinical characteristics.

Variable	All patients (n = 28)	Survivors (n = 21)	Non-survivors (n = 7)	p-value
Age (SD)	60 (13)	62 (11)	58 (11)	0.42
Race (%)				
Caucasian	16 (57)	12 (57)	4 (57)	0.83
Black American	11 (39)	8 (38)	3 (43)	
Other	1 (4)	1 (5)	0 (0)	
Ethnicity (%)				
Non-Hispanic	24 (100)	19 (100)	5 (100)	0.99
Hispanic	0 (0)	0 (0)	0 (0)	
Sex (%)				
Male	16 (57)	13 (62)	3 (43)	0.38
Female	12 (43)	8 (38)	4 (57)	
Preexisting conditions (%)				
Hypertension	20 (71)	14 (67)	6 (86)	0.33
Coronary artery disease	2 (7)	2 (9)	0 (0)	0.40
Chronic heart failure	7 (25)	3 (14)	4 (57)	0.02
Diabetes mellitus	6 (21)	4 (19)	2 (29)	0.59
End stage renal disease	5 (18)	3 (14)	2 (29)	0.39
Malignancy	5 (18)	5 (24)	0 (0)	0.15
Vital signs (IQR) *				
Heart rate (beats / min)	100 (89, 111)	100 (91, 113)	102 (82, 105)	0.54
Respiratory rate (breaths / min)	18 (18, 21)	18 (18, 20)	18 (18, 29)	0.68
Systolic blood pressure (mmHg)	105 (91, 114)	105 (92, 110)	100 (75, 128)	0.60
Diastolic blood pressure (mmHg)	55 (48, 64)	56 (50, 63)	49 (38, 65)	0.18
Baseline Laboratory Values (IQR) *				
White blood count (x 1,000 cells/mm^3)	15.7 (7.2, 24.8)	16.8 (6.7, 26.0)	13.0 (7.4, 19.3)	0.79
Hemoglobin (mg/dL)	10.3 (9.1, 12.2)	10.8 (8.8, 12.4)	10.0 (9.5, 10.3)	0.36
Platelet count (x 1,000 cells/mm^3)	194 (134, 244)	194 (140, 215)	154 (121, 338)	0.77
Creatinine (mg/dl)	2.1 (1.4, 3.6)	1.9 (1.2, 3.2)	2.3 (2.1, 4.8)	0.16
Total Bilirubin (mg/dL)	0.5 (0.3, 2.1)	0.5 (0.3, 0.8)	0.9 (0.3, 3.9)	0.20
Glucose (mg/dL)	117 (100, 170)	126 (95, 173)	106 (100, 127)	0.50
Disease severity (IQR) *				
SOFA score (enrollment)	8.5 (6, 10)	8 (6, 10)	9 (6, 16)	0.56
Lactate, mmol/L (enrollment)	2.3 (1.2, 3.5)	2.6 (1.3, 3.4)	2.2 (1.2, 5.1)	0.91
Treatments				
Intravenous fluids (0–6 hours, L) *	3.1 (1.8, 3.8)	3.1 (2.0, 3.6)	2.3 (1.0, 4.0)	0.43
Mechanical ventilation (%)	14 (50)	11 (53)	3 (43)	0.66

Variable	All patients (n = 28)	Survivors (n = 21)	Non-survivors (n = 7)	p-value
Vasopressor use (%)	20 (71)	14 (67)	6 (86)	0.33
Aspirin (within 24 hours)	3 (1)	2 (10)	1 (14)	0.72
Clopidogrel (within 24 hours)	0 (0)	0 (0)	0 (0)	0.99
Platelet transfusion	0 (0)	0 (0)	0 (0)	0.99