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14. ABSTRACT

Traumatic injury is a leading cause of respiratory failure, where 2-4% of persons with trauma develop acute lung injury/ acute respiratory distress syndrome (ALI/ARDS). Approximately one-third of the patients progress to ALI/ARDS, and an estimated mortality rate of 40% is seen in trauma-associated ALI/ARDS. Furthermore, in ARDS, Acute Kidney Injury (AKI) is the most frequent organ failure affecting almost 50% of the patients, increasing the mortality rate. Therefore, understanding the molecular differences between the survivor and non-survivors of ALI and developing biomarkers to predict ALI progression are urgently needed.

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1. INTRODUCTION

Traumatic injury is a leading cause of respiratory failure, where 2-4% of persons with trauma develop acute lung injury/ acute respiratory distress syndrome (ALI/ARDS). Approximately one-third of the patients progress to ALI/ARDS, and an estimated mortality rate of 40% is seen in trauma-associated ALI/ARDS. Furthermore, in ARDS, Acute Kidney Injury (AKI) is the most frequent organ failure affecting almost 50% of the patients, increasing the mortality rate. Therefore, understanding the molecular differences between the survivor and non-survivors of ALI and developing biomarkers to predict ALI progression are urgently needed.

2. KEYWORDS

ACUTE LUNG INJURY, ACUTE RESPIRATORY DISTRESS SYNDROME, SEPSIS, MITOCHONDRIA, MITOCHONDRIAL DYSFUNCTION, METABOBOLOMICS, LIPIDOMICS, BIOMARKERS

3. ACCOMPLISHMENTS

a. What were the major goals of the project?

The project aims to evaluate the multi-omic response to traumatic and septic shock with respect to acute lung injury and define mechanistically, via multi-omics, the therapeutic potential of Orai1 and/or MCU as targets for control of pulmonary vascular inflammation during shock. Initially, metabolomics and lipidomic analysis from serum was employed to determine the omics profile in the porcine ALI model where several metabolites related to mitochondrial TCA cycle and 10 classes of lipids alterations were found. Additionally, we are reporting more omics analysis done in the serum in predicting the risk factor for mortality and utilization of extra-pulmonary markers such as Blood Urea Nitrogen (BUN) and metabolites in predicting mortality in the porcine ALI model. Currently, analysis on spatial metabolomics in both kidneys and lungs are being conducted, and organic acid metabolomics will be completed for Aim 3.

Additionally, we have established ALI mouse model which will allow us to complete major task in Aim1 and Aim 2 to test the hypothesis related to endothelial mitochondrial function. Lung endothelial cells (ECs) derived from mice lacking Orai1 and MCU in ECs ($Orai1^{\Delta EC}$ and $MCU^{\Delta EC}$ mice, respectively) with appropriate floxed and cre- controls were employed. N of 6 was used for the control and n of 4-5 for Orai1 and MCU knockout. Mice were challenged with 10mg/kg for 24 hours where urine and plasma along with lung, kidney, liver and spleen were collected.

b. What was accomplished under these goals?

Metabolomics analysis was conducted on the porcine acute lung injury (ALI) model. Using pathway analysis, our previous metabolomics data on organic acids indicated a progressive increase in metabolites corresponding to amino acid catabolism. Therefore, serum was employed to analyze amino acid metabolites using the Zip-Chip platform for mass spectrometry. In this model, serial blood samples acquired at baseline and then at 3, 6, 9, 12, 18, and 24 hours post-trauma were utilized.

Previously we had reported a PCA plot indicating clear stratification of metabolites in survivors and non-survivors at 3h post-injury. Therefore, to understand the molecular differences between the survivor and non-survivors of ALI using amino acid metabolites, a Cox proportional hazard analysis was employed to quantify the association of survival with the metabolite concentration change at 3 hours post-trauma relative to baseline and summarized by the hazard ratio (HR) metric. The 2 groups are partitioned based on the mean metabolite change at 3 hours.

The analysis demonstrated that the group with increased tryptophan concentration change at 3 hours post-trauma survive significantly with higher rate than the group with a decreased change, indicating a poor outcome of survivability (Figure 1A). Therefore, a decrease in serum tryptophan level is a strong risk factor for mortality in the trauma-induced porcine model. Tryptophan metabolism has an intrinsic relationship to mitochondrial function[1,2], further confirming our previous metabolomics data that mitochondrial function is closely involved with lung injury and potentially with mortality. Additionally, we looked into a baseline measurement of the metabolites, and an increased tryptophan concentration had a better chance of survival than the group with the reduction of tryptophan level (Figure 1B). Therefore, we sought to look into an extra-pulmonary organ such as a kidney to test whether markers of kidney injury in addition to metabolites would predict mortality. Serum blood urea

nitrogen (BUN) was measured, and the baseline BUN was correlated with the baseline tryptophan level using a linear model.

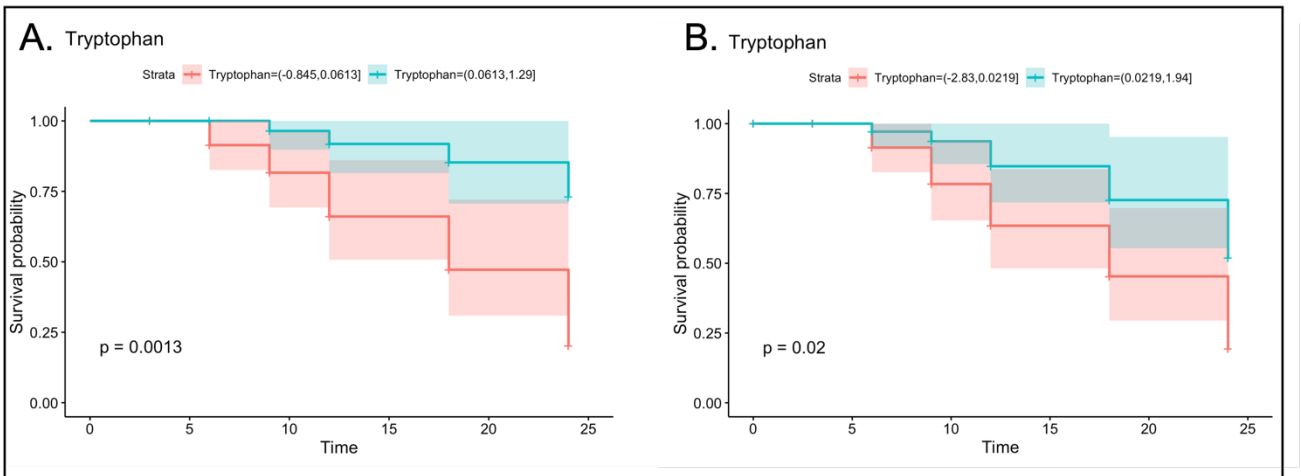

Figure 1: Cox proportional hazard ratio indicating a significant decrease in tryptophan level leads to poor outcome of survivability at 3-hour post-injury (A) and at the baseline (B)

When associating tryptophan level with the BUN, there is an opposite trend between survivors and non-survivors. In the survivors, higher tryptophan is positively associated with increased BUN. (Figure 2 A) In contrast, in the non-survivors, a negative correlation indicates that lower tryptophan coupled to high BUN increases the risk of mortality (Figure 2 B). Additionally, the linear regression model showed a significant association of tryptophan and BUN with survivors and non-survivors, therefore strengthening the correlation of BUN and tryptophan among these two groups. (Figure 3).

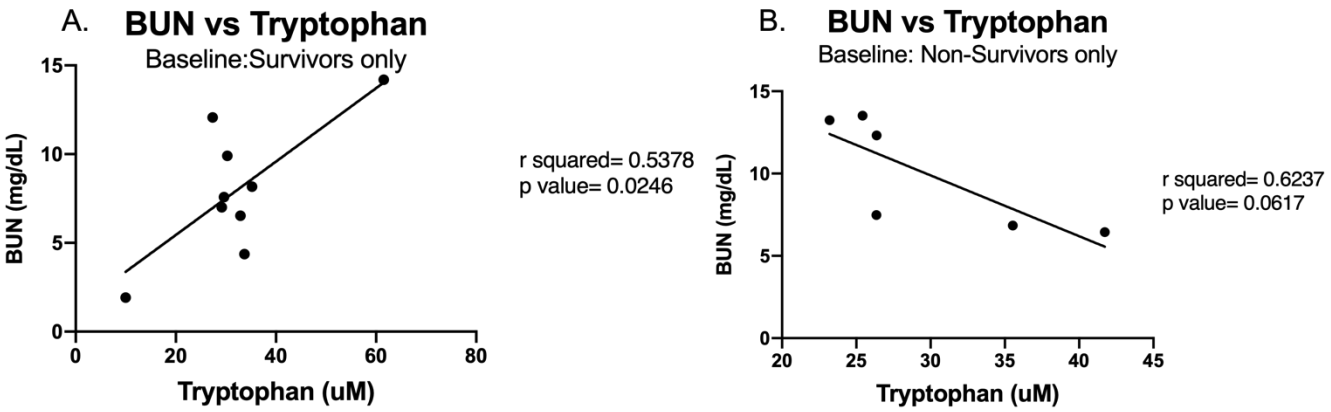

Figure 2: Stratified survivor and non-survivor groups and compared BUN and tryptophan correlation. Higher tryptophan is positively correlated to increased BUN (A). In the non-survivors, there is a negative correlation indicating that lower tryptophan coupled to high BUN increases the risk of mortality (B).

Our current results from survival analysis indicate that a decrease in serum tryptophan level is a strong risk factor for mortality. Furthermore, a combination of BUN and tryptophan could improve

mortality risk prediction in early time-point in a trauma-induced model. Therefore, complimenting our previous report that an alteration in the mitochondrial function may underlie trauma-induced ALI/ARDS progression.

Herein, we report that we have accomplished significant work from major task 1 of specific Aim 3, solidifying the findings to move forward with the major task 2, which is to test a novel interventional approach in pig trauma-induced ALI model. Metabolomics, lipidomics, and mass spectrometry imaging approach will be employed in blood, urine, and tissue samples from control and a potent cytosolic calcium blocker BTP2 treated model. Additionally, we will determine the MCU oxidation from these samples along with multi-omics analysis.

```
Call:
lm(formula = BUN.Baseline ~ Tryptophan + non.sur + cross, data = df)

Residuals:
    Min      1Q  Median      3Q     Max
-3.9170 -1.2298  0.1404  0.9905  5.1038

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  1.31053    2.37602   0.552  0.59227
Tryptophan   0.20678    0.06878   3.006  0.01195 *
non.sur     19.66222    5.41524   3.631  0.00395 **
cross       -0.57619    0.17378  -3.316  0.00689 **
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 2.582 on 11 degrees of freedom
Multiple R-squared:  0.6011,    Adjusted R-squared:  0.4923
F-statistic: 5.524 on 3 and 11 DF,  p-value: 0.01465
```

Figure 3: A linear regression model showed a significant association of tryptophan and BUN with survivors and non-survivors.

c. What opportunities for training and professional development has the project provided?

   i. Poster presentation of the project was accepted by a graduate student for the MHSRS conference in August 2021.

   ii. Another poster presentation accepted at American Society of Nephrology (ASN) by a graduate student for October 2021.

d. How were the results disseminated to communities of interest?

   i. Nothing to Report

e. What do you plan to do during the next reporting period to accomplish the goals?

Our next immediate goal is to complete analysis of organic acid metabolomics in all ALI porcine serum and spatial metabolomics in selected samples using mass spectrometry imaging in lungs and kidneys. We have completed organic acid metabolomics in all ALI samples and spatial metabolomics in representative samples on the kidney. Currently in the process of analysis from these experiments and will be starting spatial metabolomics on lung tissue. Additionally, we plan to perform metabolomics and lipidomic on our established murine sepsis-induced ALI model,

including control, Orai1, and MCU knockout in the vascular endothelial cells. The goal is to identify intracellular and extracellular signatures of mitochondrial function, EC injury, and barrier dysfunction.

IMPACT

f. What was the impact on the development of the principal discipline(s) of the project?

Traumatic injury is the leading cause of ICU admissions, with no current biomarkers to identify those at risk for common complications. There are direct and indirect causes of traumatic injury in the military setting and civilian life. Respiratory failure from septic shock is a common complication of severe trauma where 2-4% of individuals with trauma develop ALI. A key feature in trauma-associated ALI/ARDS and sepsis is the impairment of pulmonary vascular integrity, where sepsis leads to activation of innate immune cells and endothelial cells by endotoxins resulting in inflammation and oxidative stress. However, the mechanism leading to sepsis-induced ALI remains elusive.

Our omics data from lipidomic and metabolomics data reflect an alteration in the mitochondrial functions. One of the key points was to stratify survivors and non-survivors from the porcine trauma model. Understanding the molecular differences between the survivor and non-survivors of ALI will allow us to identify a molecular signature, a key biomarker that can identify those at risk for progression.

g. What was the impact on the other discipline?

Nothing to Report

h. What was the impact on technology transfer?

Nothing to Report

i. What was the impact on the society beyond science and technology?

Traumatic injury accounts for nearly half of all deaths in civilian life and costs nearly 670 billion in the United States in 2013. Currently, there is no better marker that allows to predict the progression of the injury to acute lung injury or multiple organ injury (MOF). Our research on murine and porcine ALI/ARDS model studies in combination with multi- omics data allows to find new biomarkers and therapeutic targets for trauma associated ALI and MOF.

4. **CHANGES/ PROBLEMS**

Nothing to Report

5. **PRODUCTS**

a. **Poster Presentation**

i. American Society of Nephrology, Kidney Week virtual poster presentation. "INTERACTION OF BUN AND TRYPTOPHAN IN PREDICTING MORTALITY IN TRAUMA-INDUCED MODEL", October 29, 2021.

b. **Poster Presentation**

i. Military Health System Research Symposium (MHSRS), Poster Presentation Accepted. "SERUM METABOLOMICS AS A RISK FACTORS FOR MORTALITY IN EXPERIMENTAL ACUTE LUNG INJURY". August 2021.

6. **PARTICIPANTS AND OTHER COLLABORATING ORGANIZATIONS**

a. **Nothing to Report**

7. **SPECIAL REPORTING REQUIREMENTS**

a. **Nothing to Report**

8. **APPENDICES**

a. **ASN 2021 Abstract**

b. **MHSRS 2021 Abstract**

a. INTERACTION OF BUN AND TRYPTOPHAN IN PREDICTING MORTALITY IN TRAUMA-INDUCED MODEL

Interaction of BUN and Tryptophan in predicting mortality in trauma-induced model

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Background: Multiple Organ Failure (MOF), often precipitated by Acute Respiratory Distress Syndrome (ARDS) brought on by trauma-induced injury, is a significant cause of death in military and civilian life. Furthermore, in ARDS, Acute Kidney Injury (AKI) is the most frequent organ failure affecting almost 50% of the patients, increasing the mortality rate. Therefore, understanding the molecular difference between survivors and non-survivors can significantly reduce the mortality burden.

Method: A porcine MOF model (n =17) was developed using pulmonary contusion injury at Dr. Batchinsky's laboratory. In this model, n=10 are survivors, and n=7 are non-survivors with mortality at 3, 6, and 9 hours. Serum was employed for Amino acid metabolites using the Zip-Chip platform for mass spectrometry. A Cox proportional hazard analysis was employed to quantify the association of survival with the metabolite concentration. Serum blood urea nitrogen (BUN) was measured using the assay kit, and baseline BUN was correlated with baseline tryptophan level using a linear model.

Results: In survival analysis, survivors and non-survivors were partitioned by the mean metabolite concentration. The group with increased tryptophan concentration had a better chance of survival than the group with a reduction of tryptophan from the baseline. Furthermore, when associating the tryptophan level with the BUN, there is an opposite trend between the two groups. In the survivors, higher tryptophan is positively associated with increased BUN, whereas in the non-survivors, there is a negative correlation indicating that lower tryptophan coupled to high BUN increases the risk of mortality. Additionally, linear regression model showed a significant association of tryptophan and BUN with survivors and non-survivors.

Conclusion: Survival analysis indicated that a decrease in serum tryptophan level is a strong risk factor for mortality. Since tryptophan metabolism is associated with renal failure in AKI settings, we investigated serum tryptophan association with BUN. Non-survivors have a strong negative association of tryptophan with BUN, suggesting that combination of BUN and tryptophan could improve mortality risk prediction in early time-point in trauma-induced model.

b. SERUM METABOLOMICS AS A RISK FACTORS FOR MORTALITY IN EXPERIMENTAL ACUTE LUNG INJURY

Serum Metabolomics as a risk factors for mortality in experimental acute lung injury

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Background: Traumatic injury is a leading cause of respiratory failure, where 2-4% of persons with trauma develop acute lung injury/ acute respiratory distress syndrome (ALI/ARDS). Approximately one-third of the patients progress to ALI/ARDS, and an estimated mortality rate of 40% is seen in trauma-associated ALI/ARDS. Understanding the molecular differences between the survivor and non-survivors of ALI and developing biomarkers to predict ALI progression are urgently needed.

Method: A porcine traumatic lung injury model was developed with pulmonary contusion injury. 17 pigs were employed in this study. Serial blood samples were acquired at baseline then at 3, 6, 9, 12, 18, and 24 hours post trauma. Amino acid metabolite concentration was measured using the Zip-Chip platform for mass spectrometry at each time point. A Cox proportional hazard analysis was employed to quantify the association of survival with the metabolite concentration change at 3 hours post-trauma relative to baseline and summarized by the hazard ratio (HR) metric. The 2 groups are partitioned based on the mean metabolite change at 3 hours. Metabolites with significant ($p<.05$) difference between the survival

curves were selected.

Results: 5 metabolites are significantly associated with mortalilty within 24 hours of ALI: arginine (HR=0.19, 95%CI[0.044, 0.084], p=0.028), serine (HR=0.10, 95%CI[0.018, 0.56], p=.0091), homoserine/threonine (HR=0.12, 95%CI[0.02, 0.73], p=0.021), tryptophan (HR=0.79, 95%CI[0.021, 0.023], p=0.00018), aspartic acid (HR=2.1, 95%CI[1.3, 3.4], p=0.0021). When 2 groups are partitioned by the mean metabolite concetration among these selected metabolites, we find that the group with increased tryptophan concentration change at 3 hours post-trauma survive significantly (p= 0.0013) with higher rate than the group with a decreased change.

Conclusion: Survival analysis using cox proportional-hazard analysis indicated that a decrease in serum tryptophan level is a strong risk factor for mortality in the trauma-induced porcine model. Tryptophan metabolism has an intrinsic relationship to mitochondrial function, further confirming our previous metabolomics data that mitochondrial function is closely involved with lung injury and potentially with mortality. We conclude that early measurements of tryptophan could predict mortality with acute lung injury.

Learning objective 1: Describe findings on blood biomarkers in predicting mortality for acute lung injury.

Learning objective 2: Discuss therapeutics to reduce the incidence and/or severity of ALI/ARDS and/or other lung injury secondary to trauma, transfusion, infection, burns, hemorrhagic shock, inhalation, and/or oxygen exposure.

Learning objective 3: Present the findings on an important role of mitochondria and biomarkers that reflect mitochondrial health and its potential as a new therapeutic target for acute lung injury.

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