

Abstract Title: Analysis of 572 Lung Transplants at University of Florida from 2013 to 2023: Comparing Characteristics and Outcomes of Patients with (n=38) and without (n=470) COVID-19

Hazel Hutchinson*,
Mindaugas Rackauskas, MD,
Yuriy Stukov, MD,
Anson Wang, BS,
Jin Choi, BS,
Ahmet Bilgili, BS,
Liam Kugler, BS,
Kayla Lucas, BS,
Matthew Purlee, BS,
Omar M. Sharaf, BS,
Dan Neal, MS,
Jeffrey P. Jacobs, MD

Division of Thoracic Surgery

Abstract

Introduction: For patients living with end-stage lung disease, lung transplantation is a life-saving option. During the COVID-19 pandemic, patients with irreversible lung damage from the virus were admitted due to respiratory failure. Lung transplantation was the only option for patients with irreversible COVID-19 damage who could not be weaned from extracorporeal membrane oxygenation (ECMO) or mechanical ventilation. The purpose of this study is to compare the characteristics and outcomes of those patients who underwent lung transplantation at our institution with (n=38) and without (n=470) COVID-19 related lung disease.

Methods: The purpose of this study is to compare the characteristics and outcomes of those patients who underwent lung transplantation at our institution with (n=38) and without (n=470) COVID-19-related lung disease.

Results:

- COVID patients, in comparison to non-COVID patients, were more likely to:
- Be younger (50.4±7.95 vs 60.6±11.5 years, P<0.0001),
- Be on ECMO prior to transplant (89.5% [n=34/38] vs 4.3% [n=20/470], P<0.0001),
- Be on a ventilator prior to transplant (81.1% [n=30/38] vs 3.0% [n=14/470], P<0.0001),
- Undergo lung+kidney transplant (7.9% [n=3/38] vs 0.2% [n=1/470], P<0.005),
- Have intra- or post-operative complications (100% [n=38/38] vs 63.0% [n=296/470], P<0.0001), and
- Be on ECMO post-transplant (86.8% [n=33/38] vs 22.4% [n=105/470], P<0.0001).
- One-year post-transplant survival was 100% in COVID patients and 92.66 (90.28 - 95.11) in patients without COVID, and
- Two-year post-transplant survival was 96.00 (88.62 - 99.9) in COVID patients and 83.89 (80.44, 87.50) in patients without COVID (Figure 1 p.004).

Conclusions: Survival at one year after lung transplantation is roughly similar in patients with and without COVID-19, however a 2-year survival was better in COVID patients compared to patients without COVID. A propensity matched analysis comparing patients with and without COVID-19 is a logical next step in this analysis.

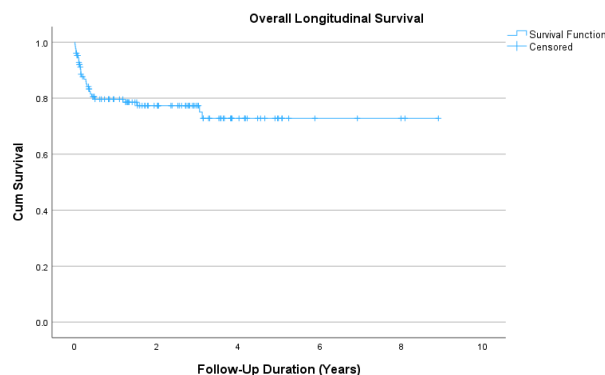
A Single-Center Experience of Aortic Root Replacement with the Freestyle Prosthesis

Iverson E. Williams*, Omar M. Sharaf, Zaid Abu-Mowis, William Ricks, Eric I. Jeng, John R. Spratt, Tomas D. Martin, Thomas M. Beaver

Division of Cardiovascular Surgery

Introduction: Aortic root replacement (ARR) with a stentless valve is effective for aortic root aneurysms, dissections, and endocarditis. Stentless valves enable a greater effective orifice area and have a malleable nature, which helps in positioning in the presence of a scar, from a prior sternotomy, or aortic root abscess. The purpose of this study is to evaluate contemporary outcomes of patients who underwent ARR with the Medtronic Freestyle heterograft prosthesis (Medtronic, Minneapolis, MN, USA). **Methods:** Single-center retrospective review of adult patients (≥ 18 years) undergoing ARR with a Medtronic Freestyle prosthesis at a University Aortic Center (7/1/2014-5/15/2023). Descriptive statistics were performed. Continuous variables are reported as mean \pm standard deviation or median (interquartile range [IQR]), and categorical variables are reported as frequency and percentage. **Results:** During the study period, 127 patients underwent a Freestyle aortic root replacement. Most patients were male (61.4%, $n=78$), with a mean age was 60.1 ± 15.2 years. Many patients had at least one prior sternotomy (80.3%, $n=102$). Other comorbidities included hypertension in 74.8% ($n=95$), diabetes in 33.9% ($n=43$), preoperative acute kidney injury in 8.7% ($n=11$), chronic kidney disease in 18.9% ($n=24$), and history of stroke in 26.0% ($n=33$). Most patients were indicated for aortic root replacement due to endocarditis (47.2%, $n=60$). Mean valve size implanted was 26.0 ± 2.2 mm. Cardiopulmonary bypass time was 312.5 ± 103.4 minutes and cross-clamp time was 220.5 ± 70.7 minutes. Postoperatively, 6.3% ($n=8$) required permanent dialysis, 9.5% ($n=12$) had pneumonia, 5.5% ($n=7$) had a stroke, and 37.0% ($n=47$) required a new permanent pacemaker. Ten (7.9%) patients died before hospital discharge. During follow-up, 3 (2.4%) patients developed root pseudoaneurysms. Follow-up echocardiography was available in 54 (42.5%) patients at a median follow-up time of 213 (IQR 49.3-525) days. The peak gradient was 21.8 ± 9.8 mmHg, and the mean gradient was 9.5 ± 4.5 mmHg. Longitudinal survival is shown in Figure 1. **Conclusions:** Among high-risk patients undergoing ARR, the Freestyle prosthesis is associated with acceptable morbidity and high freedom from reoperation.

Figure 1. Overall longitudinal Kaplan-Meier survival for the entire cohort.



No disclosures or funding sources.

Cognitive Impairment is Common and Unrecognized among Vascular Surgery Patients

Emily J Draper*, Tam B Nguyen, Amin A Mirzaie, Dan Neal, MS, Salvatore T Scali, MD, Thomas S Huber, MD, PhD, Scott A Berceci, MD, PhD, Gilbert R Upchurch, Jr., MD, Samir Shah, MD, MPH

Division of Vascular Surgery

Introduction: Patients' capacities to understand and act upon healthcare information is crucial to decision-making and high-quality care. Cognitive impairment (CI) has been associated with adverse outcomes across a range of diseases and surgeries. There is little information on its prevalence and severity in United States vascular surgery patients. We conducted a prospective observational study to better understand CI in a contemporary vascular surgery practice.

Methods: We enrolled 103 outpatients attending a vascular surgery clinic. Subjects completed a demographic survey and the Montreal Cognitive Assessment (MoCA). Chart review was used to assess comorbidities. The MoCA is a validated tool consisting of tasks such as clock drawing for assessing CI. Scores between 0-9, 10-17, 18-25, and 26-30 indicate severe, moderate, mild, and no CI respectively. Statistical analysis was performed using SAS.

Results: Our analysis included 100 patients who completed the MoCA. The average age of the entire cohort was 64.1 years. 59.4% were male, and 79% were white. The mean MoCA score was 22.6 (mild CI). 79% had CI: 69% with mild and 10% with moderate CI. Fewer years of education ($p=.007$) and hypertension ($p=.004$) had significant univariate associations with CI. There was no statistically significant difference between the primary vascular diagnosis and CI ($p=0.07$). A limited multivariable model demonstrated that years of education (OR 0.75 per year, $p=.005$) were significantly associated with risk of CI but age (OR 1.03 per year, $p=.069$) was not.

Conclusions. We found that nearly 4 out of every 5 vascular surgery patients have undiagnosed cognitive impairment. Furthermore, we found that more years of education was associated with a reduced likelihood of CI. Future work should focus on enrollment of a larger cohort and examining the impact of CI on mortality, length of stay, and other outcomes.

Table. Patient Characteristics by Impairment Status

	No Impairment (N=21, 21%)	Cognitive impairment (N=79, 79%)	p-value
Mean Age, years	58.9 (± 15.2)	65.7 (± 13.5)	.055
Formal Education, years	15.0 (± 2.8)	13.0 (± 2.5)	.007
Comorbidities			
Diabetes	6 (28.6%)	27 (34.2%)	.795
Hypertension	12 (57.1%)	69 (87.3%)	.004
Congestive heart failure	2 (9.5%)	26 (32.9%)	.053
Vascular Diagnosis Category			
Peripheral arterial disease	8 (20.0%)	32 (80.0%)	.429
Abdominal aortic aneurysms	3 (16.7%)	15 (83.3%)	
Cerebrovascular	3 (30.0%)	7 (70.0%)	
Venous	1 (12.5%)	7 (87.5%)	
Dialysis	5 (41.7%)	7 (58.3%)	

Funding Sources: RedCap, National Institutes of Health T35 Grant (NIH T35HL007489).

Extent of Burn Injury and Associations with Early Myeloid and Lymphoid Activation in Pediatric Burn Patients

Valerie E. Polcz*, Jaimar C. Rincon, Evan L. Barrios, Philip A. Efron, Lyle L. Moldawer, Shawn D. Larson

Division of Pediatric Surgery, Department of Surgery

Introduction: Thermal injury remains a leading mechanism in pediatric trauma. While most pediatric burns are small (<15% total body surface area [TBSA]), much of our understanding of immune changes after thermal injury are extrapolated from the adult population with larger burns and different burn mechanism. The role of myeloid and lymphoid populations in maintaining homeostasis and protective host immunity in pediatric burn patients is critically understudied. Our study seeks to determine the extent to which age and burn size influence the systemic host response, vulnerability to burn-induced immunosuppression and potential post-burn complications.

Methods: Patients ≤ 16 years old were recruited with small (<15% TBSA, n=6) or large (15-30% TBSA, n=4) burns. Healthy patients undergoing elective surgery were recruited as controls (n=2). Blood samples were at hospital admission, and weekly until discharge for burn patients. TNF α - and IFN γ -secreting cells were quantified using enzyme-linked immunosorbent (ELISPOT) assays in both unstimulated and stimulated (LPS or CD3/CD28) whole blood. PBMCs were isolated by Ficoll density centrifugation for quantification of HLA-DR expression. Proportions of myeloid-derived suppressor cells (MDSCs, %) and MDSC subsets (%) were determined via flow cytometry. Plasma cytokine levels were quantified using Luminex MagPix™.

Results: All cohorts were similar in age, sex and racial distribution (all $p > 0.05$). Mechanisms of thermal injury included scald, contact and flash burns. Mean TBSA was 8.5% and 23.2% for small and large burns. Burn size was associated with increased stimulated TNF α and IFN γ expression quantified by ELISpot ($p < 0.05$; Figure 1). Serum cytokine levels were similar immediately after burn injury and independent of burn size ($p > 0.05$). Significant differences in MDSC subpopulations were seen between control and large burn patients, with higher proportions of M- and PMN-MDSCs in large burns, and higher proportions of early MDSCs (E-MDSCs) in healthy controls ($p < 0.05$; Figure 1). Additional analysis revealed overall similar mHLA-DR expression levels across all groups immediately after burn injury ($p > 0.05$).

Conclusions: The immediate immune response to thermal injury, defined by ex vivo stimulated whole blood production of TNF α and IFN γ demonstrates a differential response by burn size in pediatric patients. Despite an increase in MDSC subsets in larger burns, increased ex vivo responsiveness of whole blood by ELISpot suggests a net activation of both adaptive and innate immunity in the immediate post-burn state.

Funding Sources: T32GM008721-25 (VEP, ELB), R21 HD107467-02 (JCR, SDL)

Ferroptosis in Lung Ischemia Reperfusion Injury: The Protective Role of Lipoxin A₄ via FPR2 and Nrf2 Pathways

Denny Joseph Manual Kollareth*, Victoria Leroy, Zhenxiao Tu, Alejandro Gonzalez, Makena Jade Woollet-Stockton, Guoshuai Cai, Timothy J. Garrett, Carl Atkinson, Gilbert R. Upchurch, Jr., Ashish K. Sharma

Division of Vascular Surgery

Introduction: Lung ischemia-reperfusion injury (IRI) is a common complication that occurs after lung transplantation (LTx), characterized by dysregulation of inflammation-resolution pathways, contributing to primary graft dysfunction (PGD) and chronic lung allograft dysfunction (CLAD). Specialized pro-resolving mediators (SPMs) are a family of bioactive molecules involved in the resolution of inflammation. In the present study, our objective was to examine whether ferroptosis (excessive iron-mediated cell death) contributes to lung IRI and to investigate the role of lipoxin A₄ (LxA₄, a bioactive SPM)-mediated signaling pathway in the mitigation of ferroptosis and resolution of lung IRI.

Methods: Single-cell RNA sequencing data of lung tissue from post-LTx patients, reported in a previous study (PMID: 36946463) was analyzed to determine the expression of ferroptosis related genes. Murine lung hilar ligation model of IRI (1hr of left lung ischemia followed by 6hr of reperfusion) or an orthotopic LTx was used with C57BL/6 wild-type (WT), Balb/c, formyl peptide receptor 2 knockout (*FPR2*^{-/-}) or nuclear factor erythroid 2-related factor 2 knockout (*Nrf2*^{-/-}) mice. Animals were intratracheally administered with saline or LxA₄ (100 ng/kg), 1hr before ischemia. To determine the role of pharmacological inhibition of ferroptosis in lung IRI, WT mice were injected with lipoxstatin-1 (50 mg/kg body weight, i.p.) 1hr before ischemia. Lipidomic analysis on left lung tissue was performed to determine the changes in oxidized and intact lipid profile. Lung function, cytokine levels (CXCL1, HMGB1, IL-17, TNF- α in BAL fluid), myeloperoxidase (MPO) expression and histology were assessed after IRI. Values are expressed as mean \pm SE and ANOVA was performed with Tukey's test to determine statistical significance ($p < 0.05$).

Results: Results from sc-RNA analysis showed alterations in the expression of key ferroptosis related genes (SLC7A11, SLC3A2, HIF1 α and ACSL4) in alveolar epithelial type II (ATII) cells indicating the involvement of ferroptosis in human CLAD. Further, lipidomic analysis showed decreased levels of intact lipid species (phosphatidylcholine (PC), phosphatidylethanolamine (PE), sphingomyelin (SM)) and increased levels of oxidized lipid species (oxPC, oxPE, ox triglycerides) in IRI compared to the sham group. Additionally, lipoxstatin-1 treated mice displayed significant attenuation of lung dysfunction (decreased airways resistance and PA pressure as well as increased pulmonary compliance) and injury (decreased CXCL1, IL-17, HMGB1 and TNF- α , neutrophil infiltration and MPO expression) after IRI compared to untreated controls. LxA₄ treatment attenuated lung dysfunction (increased pulmonary compliance: 6.3 \pm 0.2 vs. 2.6 \pm 0.08 and 4.3 \pm 0.2 μ L/cm H₂O, $p < 0.0001$), inflammation, injury and ferroptosis (GSH: 39.3 \pm 1.4 vs. 20.6 \pm 0.8 and 31.1 \pm 2.2 μ g/mg protein, $p < 0.0001$; MDA: 0.096 \pm 0.04 vs. 2.9 \pm 0.4 and 1.2 \pm 0.1 nmol/mg protein, $p < 0.0001$) in WT mice, but not in *FPR2*^{-/-} or *Nrf2*^{-/-} mice, respectively. In LTx model, LxA₄ reduced neutrophil infiltration and increased PaO₂ levels (181 \pm 7.5 vs. 76.5 \pm 5.6 mm Hg, $p < 0.0001$). Furthermore, in an *in vitro* model of IRI, LxA₄ pre-treatment protected against ferroptosis (increased GSH and Nrf2 as well as decreased MDA levels) compared to untreated ATII cells.

Conclusion: Our study demonstrates that LxA₄/FPR2 signaling alleviates ferroptosis via Nrf2 activation in ATII cells to offer protection against lung IRI.

Funding sources: This work was supported by David and Kim Raab Foundation (AKS) and National Institute of Health (NIH) RO1 HL140470-0181.

Abstract Title: Methylation-Based ctDNA Quantification: A Promising Non-Invasive Method for Cholangiocarcinoma Detection

Authors: Anastasia Chambers*, Isabella Angeli-Pahim, Sergio Duarte, Ilyas Sahin, Ali Zarrinpar

Department of Transplantation and Hepatobiliary Surgery

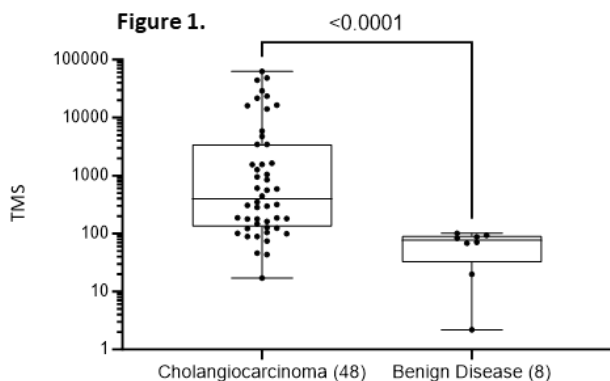
Introduction: Cholangiocarcinoma (CCA) is the second most common primary liver cancer, accounting for around 10-15% of all hepatobiliary malignancies. Although it is rare, it has an extremely poor prognosis, with a five-year survival rate of <10%. The current standard-of-care method for detection and monitoring of CCA, CA19-9, is limited by a low sensitivity of 72%. The development of a more accurate, non-invasive method of detection could facilitate early diagnosis and improve patient outcomes. Circulating tumor DNA (ctDNA) has emerged as a non-invasive method for cancer monitoring, with a novel method of quantification through methylation patterns bypassing the need for tumor biopsy. Our team demonstrated that this method to be effective in HCC. We propose that identifying and quantifying ctDNA via methylation patterns could serve as a promising method of CCA detection.

Methods: We enrolled 9 subjects undergoing systemic therapy and 4 negative control subjects with benign hepatic cysts at the University of Florida Health. Multiple blood samples were collected at various time points across treatment. Absolute quantification of methylated ctDNA molecules was conducted, evaluating multiple CpG sites on approximately 550 preselected cancer-specific amplicons. The "Tumor Methylation Score" (TMS) was calculated by measuring the difference between the number of methylated molecules in the plasma and buffy coat. A cutoff value of ≤ 120 normalized methylated molecules was established as within normal limits.

Results: We compared 56 blood samples (48 from CCA and 8 from benign cyst patients). We found that TMS accurately distinguished these two groups with an impressive significance of <0.0001 (Fig. 1). Among this cohort, TMS had a sensitivity of 81%, and a specificity of 100%, while CA19-9 had a sensitivity of 73%. There were statistically significant differences in the scores in a number of amplicons between HCC and CCA, as well as between iCCA and eCCA.

Conclusions. In conclusion, these findings demonstrate the effectiveness of ctDNA methylation scores in identifying the presence of CCA. This represents a promising initial step towards a non-invasive method for CCA detection and monitoring.

Funding Sources. CtDNA testing was performed by BillionToOne.



Abstract Title: Multimodal Nuclear Factor-Erythroid-2-Related Factor (Nrf2) Therapy In The Context Of Mammalian Target Of Rapamycin (mTOR) Inhibition Regulates Immune Reprogramming In Response To Infection Early After Burn And Inhalation Injury

Authors Matthew D. Alves, Ryan A. Clark, Denise A. Hernandez, Madelyn P. Smythe, Shannon Wallet, Benjamin G. Keselowsky, Robert Maile

Division of Acute Care Surgery

Abstract.

Introduction: Severe burn injuries induce acute and chronic susceptibility to infections, largely attributed to a hyper pro-inflammatory response followed by a chronic dysfunctional immune response. Concurrent inhalation injury induces damaging inflammation in the airway, with acutely hyperactive pulmonary macrophages and neutrophils with increased reactive-oxygen and nitrogen species (RONS) activity, unable to clear infection, but causing airway damage. Nuclear Factor-Erythroid-2-Related Factor (NRF2) is a critical immunomodulatory component, that when activated, induces protective anti-inflammatory pathways. Mammalian Target of Rapamycin (mTOR), when inhibited, reduces pro-inflammatory responses. Therapeutic use of these targets is limited, as known modulators of these pathways are saline insoluble and require long-term application. Drugs with poor aqueous solubility can be encapsulated in PLGA microparticles (MP) to enable localized delivery of the drug with an extended release profile. We have demonstrated that administering NRF2 agonist (CDDO), in addition to an mTOR inhibitory (Rapamycin, RAPA), encapsulated in MP reprogrammed the acute pulmonary hyper-inflammatory response following a mouse burn and smoke inhalation injury (BI). We hypothesized that therapy with CDDO/RAPA(combo)MP would decrease bacterial susceptibility acutely after BI.

Methods: Female C57BL/6 mice underwent sham or BI injury (n=6-8 per group), with comboMP administered i/p to one BI group 1 hour after injury. 48 hours later we i/v inoculated all mice with 4×10^6 CFU of *Pseudomonas aeruginosa* (PA01). After 48 hours, we collected and isolated lung and liver tissue and quantified infection by bacterial culture, lung and spleen mRNA for immune gene expression (nanoString and Ingenuity Pathway Analysis [IPA]), and lung tissue and plasma for cytokine analysis (Luminex).

Results: We found significantly ($*p < 0.05$) decreased bacterial CFU in the lung and liver of comboMP treated BI (mean 200CFU/g lung) and sham (mean 0CFU/g) mice versus untreated BI mice (mean 1×10^4 CFU/g). Cytokine analysis of the lung and plasma revealed a significant decrease in MCP-1 and IL-6 in comboMP-treated versus untreated BI mice. Gene analysis of lung tissue revealed significant reprogramming with significant ($*p < 0.05$) changes in immune gene expression (18 \uparrow and 11 \downarrow) in comboMP-treated versus untreated BI infected mice. IPA revealed upregulation of Macrophage Classical Activation (Z-score=+1.63), Pathogen-induced Cytokine Storm (+1.3), and downregulated IL-4 and IL-13 (-1.3), RONS in Macrophage (-1), IL-6 (-0.4) signaling pathways.

Conclusions: Our findings suggest that the multi-modal MP-based therapy holds promise in reprogramming the immune response after burn injuries, particularly by mitigating the hyper-inflammatory phase.

Funding Sources. NIH NIGMS R01 GM131124 (Maile) / NIH NIGMS R01 GM146134 (Maile)

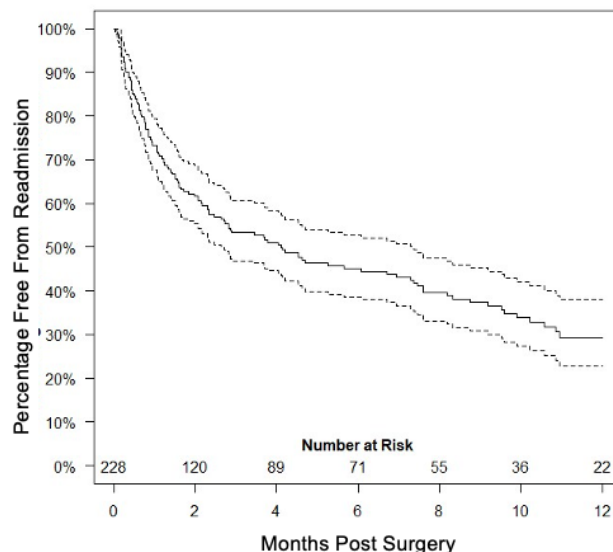
Nonwhite Race, Female Sex, and Socioeconomic Status are Associated with Increased Risk for Hospital Readmission Following Revascularization for Chronic Limb Threatening Ischemia

Walker R. Ueland MD*, Muhammad J. Javed MD, Tam Nguyen BS, Amin Mirzaie BS, Udochukwu Amanamba BS, Scott A. Berceli MD, PhD, Salvatore T. Scali MD, Martin R. Back MD, Thomas S. Huber MD, PhD, Gilbert R. Upchurch MD and Samir K. Shah MD, MPH

Division of Vascular Surgery, Department of Surgery, University of Florida

Introduction: Unplanned hospital readmission following surgery for peripheral arterial disease (PAD) is among the highest in all diagnosis-related groups. Among patients with PAD, those with the most severe form, chronic limb-threatening ischemia (CLTI), have particularly high rates of limb-loss, mortality, and readmission. Previous studies have examined risk factors for 30-day readmission following lower extremity bypass. Few studies, however, have elucidated risk factors for readmission beyond the 30-day window. Among patients with CLTI, we sought to: 1) outline rates of readmission beyond 30 days up to 1 year; 2) identify patient and procedural characteristics associated with readmission. **Methods:** Patients who underwent open or endovascular intervention for CLTI at the University of Florida were identified by CPT codes from 1/6/2020 to 5/25/2022. Demographic, operative, and one-year outcome data (including readmissions) were collected retrospectively. We used multivariable modeling to understand factors associated with readmission. **Results:** 247 patients underwent open or endovascular intervention for CLTI. One-hundred and thirty patients were readmitted within one year (53%, Figure 1). The most common indications for readmission within 30 days and one year were wound infection (43.1%) and new rest pain or tissue loss (32.3%), respectively. Nonwhite race (38 vs 14 patients, $p=0.002$), area deprivation score (70.4 vs 64.8, $p=0.04$), chronic kidney disease (CKD, $p=0.05$), acute kidney injury (AKI, $p=0.008$) and hospital length of stay (LOS, 9.4 vs 7.1 days, $p=0.013$) were associated with increased risk for hospital readmission. 67 patients (28%) required multiple hospital readmissions. Nonwhite race ($p=0.014$), female sex ($p=0.05$), AKI ($p=0.014$), and hospital LOS ($p=0.009$) were associated with multiple readmissions. Patients undergoing intervention for tissue loss were 2-3x more likely to be readmitted within 30 days and one year, respectively. **Conclusions:** Postoperative readmission among patients with CLTI is high. Nonwhite, female, and low socioeconomic status patients are at increased risk for readmission following revascularization for CLTI. CKD and AKI are risk factors for readmission, while prior revascularization and operative approach (endovascular vs. open) are not.

Figure 1. Long-Term Freedom From Readmission



Plasma Exosomal Cargo after Multicompartmental Trauma Marked by Myodegenerative miRNA

Stacey K. Drury, MD^{1*}; Julia A. Salvo¹; Kolenkode B. Kannan, PhD¹; Jennifer A. Munley MD¹; Letitia E. Bible, MD¹; Philip A. Efron MD¹; Shannon Wallet PhD²; Robert Maile PhD¹; Alicia M. Mohr, MD¹

¹Department of Surgery and Sepsis and Critical Illness Research Center, Department of Surgery, University of Florida, Gainesville, FL, USA

²Department of Oral Biology, University of Florida College of Dentistry, Gainesville, Florida

Introduction Exosomes are nanoparticles that have been linked to persistent multiorgan changes following severe injury, however, the mechanism by which these changes are communicated systemically remains unknown. In this study we sought to identify exosomal cargo which may mediate these changes with a focus on differential content of microRNA (miR) in plasma exosomes following multicompartmental trauma. *Methods* Plasma was collected from male rats (N=4/group) subjected to previously described polytrauma (PT) model including lung contusion, hemorrhagic shock, cecal ligation and pseudofracture with a corresponding injury severity score of 25. A subset were then subjected to daily restraint stress (PTRS) simulating the prolonged stress seen in chronic critical illness following traumatic injury. Exosomes were isolated at seven days post injury using the Total Exosome Isolation Kit. Size and concentration of exosomes were evaluated using Nanoparticle Tracking Analysis with ZetaView. Electron microscopy was used on a subset of samples to image exosomes as a separate method of verification. Direct quantification of miRNA was performed by ROSALIND Bioinformatics Software (Healthcare Technology Systems, San Diego, CA). Differences in rat and soleus weight were assessed using one-way ANOVA and Tukey's test for post-hoc comparisons ($p \leq 0.05$). *Results* We identified 32 miRNAs that were differentially expressed between PT and PTRS combined as compared to naïve rats ($p < 0.05$), 19 of which were downregulated relative to naïve. The top five differentially expressed were miR-296-3p, miR-501-3p, miR-485, miR-1 and miR-1197. In particular, miR-1 was elevated in the PT and PTRS groups relative to naïve. Mir-1 is selectively expressed in skeletal and cardiac muscles where it is classified as a "degenerative miRNA." In line with degenerative miR changes, PT and PTRS rats demonstrated less weight gain (PT -0.8%, PTRS 3.4%) compared to naïve rats (11.4% $p = 0.05$). Soleus weight was 30% lower following PTRS compared to naïve. *Conclusions* Plasma exosome miRNA cargo demonstrated differential expression as a result of traumatic injury and restraint stress. Elevated myodegenerative miRs were associated with muscle injury as well as restraint stress. These findings describe a potential mechanism by which traumatic injury "communicates" across organ systems via plasma exosomes and may contribute to muscle weakness during chronic critical illness.

Funding Sources: NIH Molecular Biology in Burns and Trauma T32-GM008721-25

Abstract Title: Plasma Extracellular Vesicle MiRNA Cargo And Resultant Macrophage Immune Reprogramming Are Associated With Poor Outcomes After Sepsis

Authors Arielle Ayotte, Denise A. Hernandez, Madelyn P. Smythe, Ivanna Rocha, Shannon Wallet, Ricardo Ungaro, Philip A. Efron, Lyle L. Moldawer, Robert Maile

Division of Acute Care Surgery

Abstract.

Introduction: Sepsis and septic shock have mortality rates up to 56% in developed countries. Sepsis survivors often experience persistent chronic critical illness (CCI). We have found that plasma extracellular vesicles (EVs) are key drivers of acute and chronic immune dysfunction after trauma. We hypothesized that plasma EV collected from CCI and non-CCI patients have differential a) cargo and b) immunoregulatory function both early and late after sepsis.

Methods: We obtained plasma samples from 4 healthy individuals and 12 adult sepsis patients (6 CCI [>14 days in the ICU], 6 non-CCI) collected days 1 and 7 after diagnosis (overall range of 2-60 days in the ICU). We isolated EV using size-exclusion chromatography (IZON qEV). We quantified the morphology, size and concentration of EV using electron microscopy and nanotracking analysis, and nanoString to analyze miRNA cargo. We then stimulated human THP-1 macrophages with 10^9 /ml of each EV sample, then analyzed immune gene transcription *via* nanoString (and identified significantly impacted immune pathways by Ingenuity Pathway Analysis (IPA)).

Results: At days 1 and 7, CCI-EV are significantly ($*p<0.05$) larger (median size, 133nm, suggesting microvesicle-bias) than non-CCI-EV (113nm, exosome-bias), with significantly fewer EV on day 1 in non-CCI survivors (3.07×10^{11} EV/ml) that at any other time point and patient type (median 4.2×10^{11} EV/ml). miRNA analysis revealed significant ($*p<0.05$) differences in miRNA at day 1 ($6 \uparrow$ $93 \downarrow$) and day 7 ($12 \uparrow$ $12 \downarrow$) from PICS vs non-PICS sepsis patients, encompassing changes in several well-characterized immunoregulatory miRNA (e.g. the PTEN-regulator Mir186-5p was significantly downregulated in CCI-EV versus non-CCI-EV at d7). Upon addition to macrophages, EV from day 1 and day 7 non-CCI and day 1 CCI sepsis survivors induced overall upregulation of multiple immune pathways (including IL-6, IL-1 and IL-10 signaling) versus EV from healthy subjects; in contrast, day 7 EV from CCI patients significantly ($*p<0.05$) reduced immune pathway signaling (only IL-10 and PTEN signaling were significantly upregulated). EV from day 7 non-CCI patients versus day 1 non-CCI EV significantly impacted only one immune signaling pathway (\uparrow IL-12 signaling/production). In contrast, d7 non-CCI-EV significantly ($*p<0.05$) impacted 13 immune pathways (all downregulated, including IL-12, IL-6, IL-1 signaling/production, and acute phase response signaling) in macrophages versus d1 CCI-EV.

Conclusions: These data suggest that plasma EV arise in sepsis patients with physical characteristics and miRNA cargo that may act as biomarkers to identify CCI patients. The demonstration that CCI and non-CCI-EV have differential immune reprogramming ability suggest EV are key modulators of the underlying immune dysfunction observed clinically in PICS sepsis patients.

Funding Sources. None

Pyroptosis exacerbates innate immune injury to TAADs

Muhammad Jawad Javed*, Laura Carrasco, Rachael M. Howard, Hua Li, Marvin LS. Dirain, Guoshuai Cai, Gilbert R. Upchurch Jr., Zhihua Jiang

Department of Surgery, University of Florida

Introduction: Inflammatory cell death is one of the critical cellular events occurring during TAAD (thoracic aortic aneurysms and dissections) pathogenesis. Once activated, it results in releasing of intracellular contents including DAMPs, spreading inflammation to the surrounding milieu. Among the programmed death pathways identified to date, pyroptosis and panoptosis converge on the formation of membrane pores, leading to explosive cell deaths. Gasdermin D (GSDMD) is one of the pore-forming mediators that are induced in TAADs. We hypothesized that pyroptosis plays a significant role in TAAD pathogenesis.

Methods: TAADs were induced in mice by topical application of elastase on the ascending aorta and oral delivery of BAPN. Using this model, we first evaluated effect of BAPN on TAADs with wild type mice treated with active elastase, inactivated elastase, or elastase + BAPN (n=10-15 per group). Then, we profiled temporal gene expression in TAADs and control aortas with bulk RNAseq. Ingenuity Pathway Analysis (IPA) was performed to identify activated pathways and potential upstream regulators. Furthermore, *Gsdmd*^{-/-} mice were employed to determine the effect of GSDMD on TAADs. All mice were adult (9-14 weeks of age) and on a C57BL/6J background. Serial ultrasound imaging was done to monitor aortic dilation along with necropsies to document the cause of death. Immunohistochemistry assays and histological analysis were performed to evaluate inflammation and aneurysmal degeneration.

Results: Elastase +BAPN (E+B) treated mice had significantly larger thoracic aortic diameters and rate of dilatation in comparison to elastase only and inactivated elastase treated mice at day 28 (Rx p=0.002, time p<0.001). We also found that E+B treated TAs had significantly more media loss (p=0.047) and adventitial thickening (p<0.001) as compared to inactivated elastase treated TAs. It was also associated with intima-media tears and evidence of intramural hemorrhage. RNA seq data and IPA demonstrated that at day 7, both Elastase treated TAs and inactivated Elastase treated TAs had similar upregulation of inflammatory pathways. However, at day 14, Elastase treated TAs had significantly higher upregulation of pyroptosis and innate immune response related pathways in comparison to Inactivated elastase treated TAs, whereas pathways related to the resolution of immune response were highly upregulated only in Inactivated elastase treated TAs. *GSDMD*^{-/-} mice had significantly less TA diameter and rate of dilatation, dissection events and full thickness ruptures as compared to wild type C57 mice (gene p=0.027, time p<0.001) in elastase +BAPN model at day 28. Moreover, IHC assays done on human thoracic aortic dissection samples showed cleaved Gasdermin D positive regions.

Conclusions: Our study demonstrated that pyroptosis contributes to the formation of TAADs in mice by exacerbating innate immune response and preventing the resolution of acute inflammation. Furthermore, pyroptosis occurs in human TAADs and may represent a potential therapeutic target.

Funding Sources. R01HL148019 and 1R01HL153545

Abstract Title Single Versus Multiple Truncal Endovenous Ablations in the Vascular Quality Initiative

Authors Griffin P Stinson*, BS; Jonathan R Krebs, MD; Dan Neal, MS; Salvatore Scali, MD; Nicholas H Osborne, MD, MS; Benjamin N Jacobs, MD

Division of Vascular Surgery and Endovascular Therapy

Abstract

Introduction Chronic lower extremity venous disease is widespread and increasing in prevalence. Endovenous ablation has arisen as a promising treatment for chronic venous disease. Although endovenous ablations carry a more favorable side effect profile compared to ligation or stripping, endovenous heat induced thromboses (EHIT) are still a potential complication. This study sought to compare the incidence of EHIT in single versus multivessel endovenous ablations within the Vascular Quality Initiative (VQI) database.

Methods The VQI database was queried for adult endovenous ablations. The primary reference group included all single truncal vein ablations. The comparison groups included instances of two or more ipsilateral truncal vein ablations, instances of two or more ipsilateral truncal vein ablations (at least one ablation and one other treatment), and instances of two or more truncal vein ablations on either side. Univariate and multivariate analyses were conducted.

Results A total of 29,539 procedures were included; 14,226 single ablations, 6,693 two or more ipsilateral ablations, 1,143 two or more ipsilateral ablations with other treatment, and 7,477 two or more ablations on either side. Though demographic and procedural differences existed, few were of clinical significance. There was increased incidence of EHIT when comparing single ablations (2.0%) and two or more ipsilateral ablations (1.3%, $p=0.020$), though the increased treated vein length (40 [28, 49]cm vs 28 [20, 44] cm, $p<0.0001$) associated with single ablation is a known EHIT risk factor. None of the multiple ablation comparison groups demonstrated increased incidence of any EHIT or EHIT at 30 days compared to the reference group (all $p>0.05$). There were no differences in time to EHIT diagnosis (all $p>0.05$) or any complications. Further, upon multiple logistic regression analysis, performance of multiple ablations was not associated with increased odds of 30-day EHIT for the two or more ipsilateral truncal vein ablations (OR=0.60, 95%CI (0.256-1.42), $p=0.250$), the two or more ipsilateral truncal vein ablations (at least one ablation and one other treatment) (OR=1.10, 95%CI (0.561-2.01), $p=0.852$, and the two or more truncal vein ablations on either side (OR=0.89, 95%CI (0.477-1.67), $p=0.718$) groups when compared to the single ablation group.

Conclusions Multiple venous ablations are a safe and effective treatment option for patients with chronic venous disease impacting multiple vessels and are not associated with increased incidence or odds of adverse complications or the development of EHIT.

Funding Sources None

Triple Targeting EGFR/MEK/BCL-xL Suppresses Tumor Growth via PI3K Pathway

Song Han*, Gerik W Tushoski, Jordan McKean, Alexandra J Crespin, Oguejiofor Chibeze, [Thom George, Carmen Allegra](#), Jordan McKean, Steven J. Hughes.

Division of General Surgery

Introduction. Previous studies showed that targeting MEK in KRAS mutant cancer cells resulted in resistance due to reactivation of downstream ERK kinases, possibly due to EGFR/HER pathway activation. Our hypothesis that a triplicate regimen targeting MEK, BCL-xL would be effective for PDAC, and this was proven previously in cultured patients-derived cell lines. Here, we further to test this theory using PDX *in vivo* model with mechanistic confirmation via assessment of pathway activity analysis.

Methods. We performed a short-term response assessment study in the PDAC-Im1 cell line in NOD-SCID mice. Twelve tumor-bearing mice per group were treated with a duplet (trametinib, a MEK inhibitor plus the BCL-xL PROTAC degrader, DT2216), versus a triplet (afatinib, a pan-EGFR/HER inhibitor) versus appropriate controls. Targeted MEK pathway and apoptotic signaling molecules were analyzed via ELISA multiplex. Group comparisons were analyzed using two-way ANOVAs and adjusted p-value (q value) < 0.05 were considered significant.

Results. PDX tumor regression was observed from fourth dose onwards in both duplet (49.5% reduction, q value = 0.0183) and triplet (68.1% reduction, q value = 0.0003) treatments in comparison to vehicle controls. Importantly, none of the mice experienced weight loss or other toxicity with the DT2216 PROTAC degrader. Significant reduction of tumor size between duplet and triplet group was observed at dose 5 with a measured reduction of 46.8% tumor size (q value = 0.0339), and at dose 6 (59.2% reduction, q value < 0.0001). By the end of the experiment, around 50% of PDX tumors shrank in the triplet treatment group, whilst 25% and 60% PDX tumors significantly progressed in the duplet and vehicle treatment groups, respectively. Three mice from each group were sacrificed after treatment dose 3 and tumors were collected for target pathway analysis. As expected, inhibition of MEK1 activity and downstream Erk1/2 was observed, indicated by reduced phosphorylation levels from both duplet and triplet treatments compared to no treatment controls. Importantly and supporting our hypothesis, triplet treatment further altered phosphorylation of the Erk substrate p-STAT3 and several phosphoproteins in the PI3K/AKT pathways, i.e., pAKT, pGSK, p70S6K, p90RSK, significantly differing from observations in the duplet treatment group.

Conclusions. Resistance to targeted therapy of KRAS-mutant pancreatic cancer can be mediated via EGFR/HER pathway activation. A precision, triplet regimen is well tolerated and overcomes this mechanism of resistance, and actually leads to tumor reduction in an *in vivo* model of pancreas cancer.

Whole-Blood Stimulation with LPS, BCG and Effects of Age on Inflammatory Cytokine Production in Neonates

Valerie E. Polcz*, Jaimar C. Rincon, Evan L. Barrios, Philip A. Efron, Lyle L. Moldawer, Shawn D. Larson

Division of Pediatric Surgery, Department of Surgery

Introduction: The early immune system is characterized by an immunosuppressive, tolerogenic phenotype adapted for materno-fetal tolerance prior to, and rapid colonization with commensals shortly after birth. This phenotype may also contribute to increased infectious susceptibility, sepsis and death, particularly among preterm and low birthweight infants. BCG vaccination at birth has been demonstrated to be a potent immunoadjuvant with non-specific immune effects that may confer protection against infection by unrelated pathogens; however, it is unknown how age may influence the effectiveness of BCG in stimulating adaptive and, in particular, innate immune function.

Methods: Preterm (26-36 weeks gestational age [GA]) and term infants (>37 weeks GA) were recruited in addition to healthy adults 18-45 years old. A 250-300 μ L peripheral blood sample was obtained and whole blood ELISpot assays were performed to quantify TNF α -secreting cells with stimulation via LPS (10 ng/mL), BCG (5 μ g/mL), or combination. Following 24-hour incubation, ELISPOT supernatants were harvested and cytokine levels were quantified from these samples using the Luminex MagPix™ platform.

Results: 8 healthy adults (HA), 9 full term (FT), and 9 preterm (PT) blood samples were analyzed. Sex and race/ethnicity were similar between FT and PT patients, with mean GA of 39.8 and 32.25 weeks respectively. PT patients had significantly lower APGAR (5.5 vs 7.9, $p=0.035$) and birthweight (1920 vs 3474 g, $p<0.0001$), and were more likely to be born via C-section (72.7 vs 22.2%, $p=0.025$). Cytokine analysis showed that BCG stimulation and LPS co-stimulation with BCG led to higher overall concentrations of select pro-inflammatory cytokines, including TNF α , IL-12p70, and CXCL8 compared to unstimulated or LPS-only stimulated samples, regardless of age. IFN γ production was significantly reduced across all experimental groups in preterm compared to healthy adults ($p<0.05$, Figure 1). Cytokine production in response to LPS stimulation alone showed GA-dependent differences for IL-6, IL-8, IL-12p70, IL-23, and TNF α (all $p<0.05$).

Conclusions: Ex vivo stimulation with BCG induced notable changes in pro-inflammatory cytokine concentrations in whole blood ELISpot, with both age-dependent and independent changes noted. Findings suggest that prematurity is associated with reduced innate immune responses that can be partially restored by BCG exposure.

Funding Sources: T32GM008721-25 (VEP, ELB), R01 GM143143-01A1 (JCR, SDL)