55th Annual Midwest Student Biomedical Research Forum

Saturday, March 2, 2024

P-065 OSCILLATORY DYNAMICS SERVING VERBAL WORKING MEMORY DIFFER IN PEOPLE WITH HIV
Presenter: Kellen McDonald, Creighton University

P-067 TARGETING INFILTRATING LUNG MONOCYTE-MACROPHAGES TO REDUCE ENVIRONMENTAL EXPOSURE-INDUCED LUNG DISEASE
Presenter: Grace Moravec, UNMC

P-072 INDIVIDUALIZED CARE PLANS FOR PATIENTS WITH HIGH INPATIENT UTILIZATION
Presenter: Ashlyn O'Leary, UNMC

P-073 POTENTIAL ROLE OF RACK1 IN SUPPRESSING TRANSLATION OF FULL LENGTH RAG PROTEINS
Presenter: Tamara Olson, Creighton University

P-075 ROLE OF RAB5 MEDIATED MICROLIPOPHAGY ON HEPATOCELLULAR CARCINOMA PROGRESSION AND THERAPEUTIC DEVELOPMENT
Presenter: Kelly Otakhor, UNMC

P-076 THE RELATIONSHIP BETWEEN NEONATAL ANTHROPOMETRIC MEASUREMENTS AND BODY COMPOSITION IN TERM INFANTS
Presenter: Megan Ott, UNMC

P-077 POST-TRANSLATIONALLY MODIFIED FIBRINOGEN ACTIVATED MACROPHAGES DRIVE THE EXPRESSION OF FIBROTIC GENES IN HUMAN LUNG FIBROBLASTS
Presenter: Tanner Ourada, UNMC

P-078 PREDICTIVE MODELING WITH CLINICAL AND LABORATORY VALUES IN NEONATES WITH HYPOXIC-ISCHEMIC ENCEPHALOPATHY
Presenter: Ashley Pak, Creighton University

P-080 THE IMPACT OF LIMB FLEXION ON THE BIOMECHANICS OF THE ARTERIES OF THE CALF IN MEN AND WOMEN
Presenter: Margarita Pipinos, UNMC

OSCILLATORY DYNAMICS SERVING VERBAL WORKING MEMORY DIFFER IN PEOPLE WITH HIV

Kellen M. McDonald[1,2], Seth D. Springer[1,3], Mikki Schantell[1,3], Ryan Glesinger[1], Lucy K. Horne[1], Hannah J. Okelberry[1], Jason A. John[1], Anna T. Coutant[1], Tony W. Wilson[1,2,3]

[1]Institute for Human Neuroscience, Boys Town National Research Hospital, Boys Town, NE
[2]Department of Pharmacology and Neuroscience, Creighton University, Omaha, NE
[3]College of Medicine, University of Nebraska Medical Center (UNMC), Omaha, NE

Background: Medical advances have greatly improved the quality of life and extended the longevity of people with HIV (PWH). However, many PWH still develop neurocognitive deficits even in the presence of effective viral suppression, which can greatly impact their ability to perform daily activities. These deficits have been linked with aberrant neuronal activity in primary sensory and higher-order executive regions. While previous neuroimaging studies have demonstrated altered neural responses during verbal working memory (VWM), the underlying temporal dynamics remain poorly understood. Thus, we used magnetoencephalography (MEG) and a VWM paradigm to quantify the underlying dynamics in PWH and matched controls.

Hypothesis: PWH will have differential alpha/beta oscillatory dynamics in language regions during a VWM task compared to control participants, and this difference in neural oscillatory activity will scale with task performance.

Experimental Design: Our sample consisted of 75 PWH and 84 controls. Participants completed a six-load VWM task during MEG. MEG measures brain function by quantifying the magnetic fields that naturally emanate from neuronal populations, providing spatially and temporally precise functional maps. Utilizing the fine temporal resolution of MEG, we were able to parse out the encoding phase of the task into four distinct time bins of 400 milliseconds each. A whole-brain repeated measures analysis was performed to assess the HIV status-by-oscillatory time window interaction across the encoding period.

Results: PWH had significantly lower accuracy on the VWM task compared to controls. We found a significant interaction effect of group by encoding window. Posthoc testing showed that controls exhibited significantly stronger alpha/beta oscillations than PWH across the entire encoding period in the left inferior temporal gyrus and prefrontal cortex, and that this effect was driven by differences in the first two windows. Further, weaker activity in the inferior temporal gyrus was significantly associated with declining accuracy across PWH and controls (p = 0.002).

Conclusions: PWH performed more poorly on the VWM task and had weaker alpha/beta activity during the encoding period in regions supporting the language component of verbal working memory. Brain-behavior relationships revealed that weaker alpha/beta oscillations in the left inferior temporal gyrus scaled with poorer task performance across all participants. Overall, these findings support the notion that HIV status modulates the neural oscillatory dynamics underlying VWM. Future work should elucidate the precise temporal dynamics of oscillations during the encoding period and quantify how key clinical indices of HIV infection modulate these dynamics.

(1) **TITLE: TARGETING INFILTRATING LUNG MONOCYTE-MACROPHAGES TO REDUCE ENVIRONMENTAL EXPOSURE-INDUCED LUNG DISEASE**

(2) **AUTHORS: Grace V. Moravec**[1], Aaron D. Schwab[1], Amy J. Nelson[1], Angela M. Gleason[1], Michael J. Duryee, Oliver Schanze[1], Debra J. Romberger[2,1], Todd A. Wyatt[2,1,3], Geoffrey M. Thiele[2], Ted R. Mikuls[2,1], Jill A. Poole[1]

(3) **AFFILIATIONS:**
[1]Department of Internal Medicine, University of Nebraska Medical Center, Omaha, NE
[2]Veterans Affairs Nebraska-Western Iowa Health Care System, Research Service, Omaha, NE
[3]Department of Environmental, Agricultural and Occupational Health, College of Public Health, University of Nebraska Medical Center, Omaha, NE

(4) **BACKGROUND**: Environmental and occupational exposures, rich in lipopolysaccharides (LPS), can reduce early allergic asthma development, but are associated with occupational asthma and chronic bronchitis. From the peripheral circulation, recruited, transitioning murine lung $CD11c^{int}CD11b^{+}$ monocyte-macrophages are robustly induced following inhalant LPS exposures, but their role in mediating lung inflammatory and pro-fibrotic lung disease is unknown.

(5) **SIGNIFICANCE OF PROBLEM:** Environmental exposure to airborne respiratory insult is often a routine component of many occupational settings, such as agriculture, military, construction, and manufacturing. As a result of acute and long-term exposure to airborne insults, fibrotic and inflammatory processes occur within the lungs, which have been associated with recruitment of monocyte-macrophage subpopulations. These processes can lead to the development and worsening of interstitial lung disease, especially when coupled with systemic autoimmune conditions, producing debilitating effects on quality of life.

(6) **HYPOTHESIS:** Reduction of systemic inflammatory monocyte-macrophages via intravenous clodronate liposome administration will result in decreased lung inflammation and fibrotic indices following environmental LPS insult.

(7) **EXPERIMENTAL DESIGN**: C57BL/6J mice were administered intravenous clodronate (vs. vehicle) liposomes to reduce circulating monocytes 24 hours prior to intratracheal instillation with 10 µg LPS or saline and euthanized 48 hours post-exposure (N=7-10 mice/group). BALF and lung tissues were collected and analyzed for mediators by ELISA, collagen content via trichrome staining, and cell determination by flow cytometry. ANOVA with post-hoc Tukey were utilized.

(8) **RESULTS**: LPS-induced lung infiltrating $CD11c^{int}CD11b^{+}$ monocyte-macrophage subpopulation was reduced by 53% ($p=0.02$) with systemic clodronate liposome administration; no other lung cell populations induced by LPS including activated resident macrophages, neutrophils, T and B lymphocytes were significantly modulated. Clodronate liposome pre-treatment reduced LPS-induced IL-6 (but not TNF-α) levels (66% reduction, $p=0.04$). There were also reductions (all $p<0.05$) in levels of LPS-induced matrix metalloproteinases (MMP)-3 (36% reduction), MMP-8 (57% reduction), tissue inhibitor of metalloproteinases (TIMP-1, 61% reduction), fibronectin (38% reduction), and collagen content (22% reduction) with clodronate vs. vehicle liposome treatment.

(9) **CONCLUSIONS**: Selective reduction of LPS-induced infiltrating lung $CD11c^{int}CD11b^{+}$ monocyte-macrophage subpopulation resulted in reduction of inflammatory, pro-fibrotic mediators, including extracellular matrix components and collagen. This suggests that targeting the infiltrating monocyte-macrophage population may reduce the adverse consequences resulting from occupational exposures to LPS-enriched inhalants.

INDIVIDUALIZED CARE PLANS FOR PATIENTS WITH HIGH INPATIENT UTILIZATION

Brady Bulian, MD[1]; Ashlyn O'Leary; Jana L Wardian, PhD, MSW[1]; Tabitha Matthias[1], MD
University of Nebraska Medical Center, Department of Internal Medicine, Division of Hospital Medicine, Omaha, NE

'High utilizers' are a small patient group who utilize a larger proportion of healthcare services resulting in much higher healthcare costs in the United States than their peers. This quality improvement project aimed to curtail utilization of hospital resources by high utilization patients via the implementation individualized care plans (ICPs). The goal of this intervention was to reduce frequency and duration of hospital admissions to improve patient health status and quality-of-life, and to reduce the total number of days of the year spent in hospital by 50%.

In 2021 at UNMC, a workgroup was assembled to find patients who would benefit from having an ICP. To do this, a report was written in Epic's reporting workbench that identified patients with more than seven hospital admissions within the previous 365 days, populating 24 patients on the initial list. The workgroup selected six patients believed to benefit from an ICP. Later, an additional six patients were included. These patients were relatively young, and their hospitalizations were assessed to be semi-elective in nature, with the potential to be managed outpatient. The vision of the workgroup was to improve quality of life and health span for patients with burdensome chronic diseases, with a mission to provide an individualized care plan (ICP) to patients who spend a sizable portion of their lives in hospital. The workgroup created an ICP which was discussed patient and placed in the electronic health record. Providers were altered to the presence of an ICP through flags in multiple locations within the chart. This accessibility to the ICP via multiple channels was key in that the guidelines for management needed to be applicable to all points of entry into the institutional system for it to achieve the goal of standardized care.

The primary chronic medical problems in these patients included sickle cell disease, chronic pancreatitis, chronic obstructive pulmonary disease, chronic adrenal insufficiency, and alcohol use disorder. The most common reasons for admission were pain, nausea with vomiting, dyspnea, and alcohol intoxication or withdrawal. A leading factor that contributed to recurrent hospitalizations and untimely demise in the cases we identified was opioid use disorder. The intervention was measured from time of ICP implementation to 365 days later. This strategy improved patient care by reducing total admissions per year from 125 to 41 (-67%); days per year in hospital from 497 to 219 (-56%); and slightly increasing days per admission from 4 to 5.4 (+26%).

By creating a general template for the ICP, it can be utilized and evaluated on a larger scale within our hospital system and beyond with the goals of improving the quality-of-life for patients, freeing up hospital beds in our facility for other patients in need, and allowing patients to experience more days in the community instead of the hospital.

POTENTIAL ROLE OF RACK1 IN SUPPRESSING TRANSLATION OF FULL LENGTH RAG PROTEINS

Tamara L. Olson, Skylar Ketteler, Patrick C. Swanson
Creighton University School of Medicine, Omaha, NE

Background and Significance

V(D)J recombination is an important process that occurs in B cells and T cells to expand the repertoire of pathogens that these cells can recognize. The Recombination Activating Gene proteins, RAG1 and RAG2, are integral to V(D)J recombination by initiating this process through binding the recognition signal sequences (RSSs) and then creating a double stranded DNA break. Work from this laboratory found that the stability of RAG1 is controlled by the $CRL4^{VprBp}$ E3 ligase, but the mechanism remains unclear. Previous work from this lab identified Receptor of Activated C Kinase 1 (RACK1) as associating with full length RAG1 (FLR1) when purified with full length RAG2 (FLR2). RACK1 is a scaffolding protein that was initially found to interact with protein kinase C to regulate transcription of cyclin D1. Subsequent studies revealed RACK1 associates with multiple factors involved in cell signaling and protein degradation pathways, as well as with the ribosome to regulate translation. Given RACK1's known function as a substrate adaptor for cullin E3 ubiquitin ligases, it was initially hypothesized that RACK1 acted as a cofactor with the $CRL4^{VprBp}$ E3 ligase to mediate RAG1 degradation. Preliminary data from the lab suggests that RACK1 does not directly regulate RAG1 stability, but when RACK1 is overexpressed, it suppresses FLR1 and FLR2 expression. Given these findings, and the known association of RACK1 with the ribosome, we investigated whether RACK1 suppresses translation of the full length RAG proteins by testing whether puromycin, which is incorporated into proteins in a manner that reflects the rate of translation, is incorporated into FLR1 or FLR2 at a lower rate when cotransfected with RACK1. These experiments helped to further understand the role of RACK1 in association with FLR1 and FLR2.

Hypothesis

Puromycin incorporation into the full-length RAG proteins will be diminished by RACK1 coexpression.

Experimental Design

HEK293T cells were transfected with maltose binding protein (MBP)-tagged full length RAG1, MBP-tagged full length RAG2, with or without FLAG-tagged RACK1. After incubation, the cells were treated with puromycin for 30 minutes. MBP-tagged full length RAG1 was purified by amylose affinity chromatography from harvested cells using amylose resin. Following purification, the clarified lysate and purified protein was detected on a western blot using antibodies specific for puromycin, MBP (8G1) and DYKDDDDK (FLAG epitope) (9A3).

Results

FLR1 was undetectable by Western blot. However, there was a 12% decrease in band value of FLR2 between the sample that was transfected with FLR1 and FLR2 without RACK1 (30 µl was loaded onto the western blot) and the sample that was transfected with FLR1 and FLR2 with RACK when 50 µl was loaded onto the western blot. There was a 25% decrease in band value between the sample with FLR1 and FLR2 without RACK1 compared to the sample with FLR1 and FLR2 with RACK when 40 µl was loaded onto the western blot.

Conclusions

Initial results showed a decrease in puromycin incorporation in FLR2 when RACK1 was co-transfected with FLR1 and FLR2. However, further studies are needed to determine the rate of puromycin incorporation by treating the HEK293T cells with puromycin for varying amounts of time. The experiments also need optimizing so that both FLR1 and FLR2 are better visualized on Western blot.

ROLE OF RAB5 MEDIATED MICROLIPOPHAGY ON HEPATOCELLULAR CARCINOMA PROGRESSION AND THERAPEUTIC DEVELOPMENT

Kelly O. Otakhor[1], Saumya Bhatt, Delia Y. Omar, Nourhan Mostafa, and Micah B. Schott

[1]University of Nebraska Medical Center, Omaha, NE

Background: Lipid Droplets (LDs) are organelles that store and mobilize neutral lipids, and their aberrant accumulation in steatotic liver disease can lead to steatohepatitis, cirrhosis, and eventually hepatocellular carcinoma (HCC), the 4th leading cause of cancer-related deaths worldwide as a result of obesity and heavy alcohol consumption. During liver disease progression, LD catabolism is majorly affected, but these mechanisms are poorly understood. Despite this correlation, the specific utilization of LDs during the tumorigenesis and growth of HCC remains inadequately understood. This is an important problem because rapidly dividing cancer cells need high amounts of energy and lipid biomass to fuel their growth and metastasis. Several proteomic studies have found Rab5, a small GTPase involved in early endosome fusion and maturation, is an LD-resident protein. Rab5 is encoded as three isoforms, Rab5A, B and C in mouse and human genomes. Preliminary results from our lab and previous studies have shown that Rab5 is implicated in the docking of early endosomes to LDs. However, the specific mechanisms by which Rab5 regulates LD trafficking and utilization in hepatocytes, particularly in a specialized form of autophagy known as lipophagy, are not well understood.

Significance: HCC is strongly associated with steatotic liver, yet the specific contribution of LDs and lysosomes in HCC remains poorly understood. Investigating the mechanisms underlying LD catabolism and lipophagy in HCC could provide valuable insights into the pathobiology of this aggressive cancer. Additionally, exploring the role of Rab5 in LD trafficking could uncover new therapeutic targets for HCC, given the connection between liver cancer and the lipid-rich environment of fatty liver.

Hypothesis: HCC tumor growth is fueled by a novel lipophagy mechanism whereby Rab5 directs endosome docking to LDs act as a signal for lysosomal fusion.

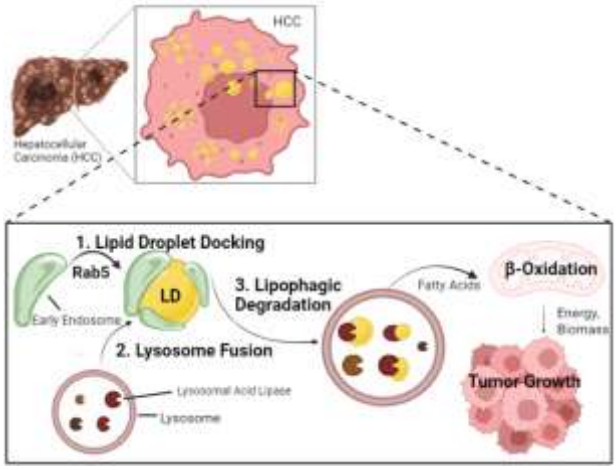


Figure 1: Schematic showing summary of my hypothesis.

Experimental design: To investigate the impact and binding patterns of Rab5-LD interaction we expressed different isoforms of GFP-Rab5 and mutants (wildtype, constitutively active Q79L, and inactive S34N) in Hep3B HCC cells invitro. To evaluate GTPase activity and Rab5-LD interaction in lipophagy, immunofluorescence and Oil Red O (ORO) staining was performed using Hep3B cells expressed with the different GFP-Rab5 isoforms and mutants under fed and starvation conditions, induced by treatment with Hank's Balanced Salt Solution (HBSS) containing calcium and magnesium ions, which stimulate lipophagy, in 1 and 4 hours, respectively. Cells were fixed and Images were taken using super-resolution microscopy. Additionally, western blot analysis of isolated LDs using density gradient centrifugation method was carried out to assess Rab5-LD association. GFP-Rab5/LD interaction was quantified using ImageJ analysis with LD-colocalization plugins.

Results: We observed that the Q79L isoform of Rab5 exhibits a more exclusive localization to LDs compared to the wildtype and S34N Rab5 isoforms suggesting that there may be a specific mechanism, possibly lipophagy, in which LDs are trafficked for degradation through a canonical Rab5 endosomal pathway. We also observe that HBSS does not appear to increase Rab5-LD interaction, or Rab5 GTPase activity.

Conclusion: Rab5 plays a role in the interaction between early endosomes and LDs. The isoforms of Rab5, both in the GTP and GDP-bound state, can colocalize to LDs. Also, Rab5 plays a role in the trafficking of LD to the lysosome. Our future experiment will focus on determining lipophagy contribution to HCC cell energy homeostasis and proliferation using seahorse metabolic analysis.

THE RELATIONSHIP BETWEEN NEONATAL ANTHROPOMETRIC MEASUREMENTS AND BODY COMPOSITION IN TERM INFANTS

Authors: Megan Ott[1], Anum Akbar[2], Matthew VanOrmer[2], Rebekah Rapoza[2], Rebecca Slotkowski[2], Allison Zetterman[1], Kayla Adams[3], Anita Zhou[1], Andrea Rodriguez Dasta[4], Teri Mauch[2], Corri Hanson[5], Ann Anderson-Berry[2], Melissa Thoene[2]

[1] University of Nebraska Medical Center (UNMC) College of Medicine, Omaha NE USA;

[2]Department of Pediatrics, University of Nebraska Medical Center, Omaha, NE 68198
[3]University of Nebraska – Lincoln, Lincoln, NE 68588
[4]University of Puerto Rico – Mayagüez, Mayagüez, PR 00681
[5]College of Allied Health Professions, University of Nebraska Medical Center, Omaha, NE 68198

Background: Infant body composition is an important tool to track growth quality and evaluate potential risk for malnutrition, obesity, or metabolic disorders. Air displacement plethysmography is the gold-standard for measuring body composition but is often cost-prohibitive. Mid-upper arm circumference, lower chest circumference, and mid-thigh circumference have been proposed as simple and cost-effective measurements of infant growth, but little is known about how well these anthropometric measurements correlate with infant body composition.

Significance: Identifying cost-effective measures of infant body composition could improve infant health through early identification and/or treatment of altered growth.

Hypothesis: Infant percent body fat will be positively correlated with anthropometric measurements.

Methods: An IRB-approved study enrolled 34 term (gestational age ≥37 weeks, 0 days) infants at the time of delivery. Birth weight, length, and head circumference percentiles were collected from infant medical records. Weight-for-length percentiles were calculated using the 2006 WHO growth standards. Mid-upper arm (n=24), lower chest (n=24), and mid-thigh (n=20) circumference were measured within the first week of life. Fat mass, fat-free mass, and percent body fat were measured by air displacement plethysmography (PEA POD). Spearman correlations were used to evaluate the relationship between anthropometric measurements and body composition.

Results: Median birth gestational age was 39.3 weeks (IQR 38.3-40.1) and 47.1% of infants were male. Percent body fat was positively correlated with birth weight percentile (rs=0.36, p=0.04) and weight-for-length percentile (rs=0.38, p=0.03). There was no correlation between percent fat and birth length (p=0.52) or head circumference (p=0.20) percentile. Similarly, there was no correlation between percent fat and mid-upper arm (p=0.40), lower chest (p=0.14), or mid-thigh circumference (p=0.08). However, fat mass and fat-free mass were both positively correlated with infant arm, chest, and thigh circumference.

Conclusion: Infant percent body fat was not correlated with mid-upper arm, lower chest, or mid-thigh circumference, suggesting that these anthropometric measurements are not an accurate measure of infant body composition. Additional research is needed in a larger cohort to identify other potential cost-effective measures of infant body composition.

POST-TRANSLATIONALLY MODIFIED FIBRINOGEN ACTIVATED MACROPHAGES DRIVE THE EXPRESSION OF FIBROTIC GENES IN HUMAN LUNG FIBROBLASTS

Tanner Ourada[1], Nozima Aripova[1], Michael J. Duryee[1,2], Carlos D. Hunter[1,2], Amy J. Nelson[3], Breanna M. Butler[1], Bryant R. England[1,2], Jill A. Poole[3], Geoffrey M. Thiele[1,2], and Ted R. Mikuls[1,2]

AFFILIATIONS:
[1] Department of Internal Medicine, Division of Rheumatology, University of Nebraska Medical Center, Omaha, NE, USA
[2] Veteran Affairs Nebraska- Western Iowa Health Care System, Omaha, NE, USA
[3] Department of Internal Medicine, Division of Allergy and Immunology, Omaha, NE, USA

***Background*:** Cellular interactions between alveolar macrophages (MΦ) and human lung fibroblasts (HLFs) contribute to excessive pro-inflammatory and pro-fibrotic responses associated with pulmonary fibrosis. Our laboratory has previously shown that antibodies to malondialdehyde-acetaldehyde (MAA) are increased in patients with rheumatoid arthritis (RA) associated interstitial lung disease (ILD). Lung tissues from patients with RA-ILD showed enhanced staining for MAA and co-localization with citrulline (CIT).

Significance of Problem: Individuals with RA face an eightfold higher risk of developing lung disease compared to the general population with interstitial lung disease being the predominant pulmonary manifestation. RA-ILD accounts for 10-20% of all RA-related deaths and remains a substantial source of morbidity among RA patients.

Problem/Hypothesis: The mechanism by which MAA and CIT-modified proteins alter cellular interactions between MΦ and HLFs has not been well delineated. Our lab hypothesized that fibrotic gene expression and activation of signaling pathways by HLFs is enhanced in response to MΦ supernatants (MΦ-SN) collected post-stimulation with MAA and/or CIT-modified fibrinogen (FIB) compared to direct stimulation with MAA and/or CIT-modified FIB.

***Experimental Design*:** Primary HLF cells were treated with MAA and/or CIT-modified FIB or with MΦ-SN collected from PMA-activated U-937 MΦ following stimulation with MAA/CIT-modified FIB. RNA was isolated 8 hours post-treatment and evaluated for the expression of 770 genes using the NanoString® Human Fibrosis Panel. The top 30 genes with the highest fold increases (compared to FIB or MΦ-SN^{FIB}) were categorized into stages (inflammation, initiation, modification, and proliferation) based on their role in the fibrotic pathway, with several genes exerting influence on multiple pathways. Additionally, HLF cells were evaluated by Western Blot for phosphorylation of signaling pathways involved in fibrosis.

***Results*:** Treatment of HLFs with MΦ-SN from FIB-modified antigens upregulated pro-fibrotic genes in all 4 pathways beyond the effects of direct antigen stimulation. For inflammatory genes, exposure of HLFs to MΦ-SN$^{FIB\text{-}MAA\text{-}CIT}$ yielded the highest mRNA fold increase. The top 5 inflammatory genes expressed following stimulation with MΦ-SN$^{FIB\text{-}MAA\text{-}CIT}$ vs. MΦ-SN^{FIB} were CD36 (9-fold), C8A (8-fold), BST2 (7-fold), BPM-10 (6-fold), MMP9 (5-fold). The top genes for initiation, modification, and proliferation upregulated in the MΦ-SN$^{FIB\text{-}MAA\text{-}CIT}$ group were; CETP (14-fold), TJP2 (7-fold), and CD36 (9-fold). HLFs treated with MΦ-SN$^{FIB\text{-}MAA\text{-}CIT}$ in comparison to MF-SN$^{FIB\text{-}MAA}$ and MF-SN$^{FIB\text{-}CIT}$ showed an increased expression of phosphorylated; (p-) JNK ($p<0.001$ vs. MF-SN^{FIB}), p-Erk1/2 ($p<0.0001$), p-Akt ($p<0.0001$), and p-FAK ($p<0.0001$) (Fig.2). Direct antigen stimulation didn't upregulate any of the signaling pathways examined.

Inflammation:

	MΦ-SN:				Direct antigens:			
Genes	FIB	FIB-MAA	FIB-CIT	FIB-MAA-CIT	FIB	FIB-MAA	FIB-CIT	FIB-MAA-CIT
CD36	1.0	5.9	3.9	9.4	1.0	1.1	2.0	1.3
C8A	1.0	6.2	5.6	7.8	1.0	-1.2	-1.5	-2.2
BST2	1.0	6.2	2.2	6.7	1.0	1.1	-1.1	-1.1
BMP10	1.0	3.6	1.7	6.0	1.0	-1.2	1.0	1.0
MMP9	1.0	4.4	3.5	4.7	1.0	1.1	1.3	-1.0

Initiation:

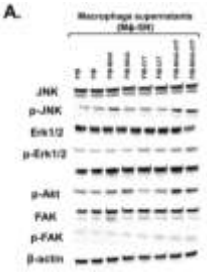
	MΦ-SN:				Direct antigens:			
Genes	FIB	FIB-MAA	FIB-CIT	FIB-MAA-CIT	FIB	FIB-MAA	FIB-CIT	FIB-MAA-CIT
CETP	1.0	6.2	4.6	14.3	1.0	-1.2	-1.6	1.2
CD36	1.0	5.9	3.9	9.4	1.0	1.1	2.0	1.3
LRP2	1.0	6.7	3.0	2.8	1.0	-7.6	-1.5	-2.3
PDGFRB	1.0	3.6	2.3	3.6	1.0	-1.3	-1.6	1.1
PLCG2	1.0	2.7	2.3	3.6	1.0	1.3	1.2	1.2

Modification:

TJP2	1.0	7.4	3.7	7.4	1.0	-1.9	-1.2	-1.4
MMP9	1.0	4.4	3.5	4.7	1.0	1.1	1.3	-1.0
VAV1	1.0	3.4	3.6	4.5	1.0	-1.1	-1.1	-1.0
COL6A3	1.0	1.9	1.8	3.3	1.0	-1.0	-1.3	-1.1
MMP10	1.0	2.4	2.0	2.9	1.0	-1.0	1.1	1.0

Proliferation:

CD36	1.0	5.9	3.9	9.4	1.0	1.1	2.0	1.3
PIK3R5	1.0	3.7	1.9	7.7	1.0	-1.2	1.4	1.5
LRP2	1.0	6.7	3.0	2.8	1.0	-7.6	-1.5	-2.3
BMP10	1.0	3.6	1.7	6.0	1.0	-1.2	1.0	1.0
MMP9	1.0	4.4	3.5	4.7	1.0	1.1	1.3	-1.0



***Conclusions*:** Our studies demonstrate that HLF interaction with MΦ released soluble mediators, particularly those initiated by exposure to dually modified antigens, is necessary to engage all 4 relevant pathways involved in fibroblast-mediated pulmonary fibrosis that characterize RA-ILD. Identification of MΦ released soluble mediators may serve as important factors of the inflammatory and fibrotic processes underlying RA-ILD pathogenesis and could represent a potential novel target for treatment.

PREDICTIVE MODELING WITH CLINICAL AND LABORATORY VALUES IN NEONATES WITH HYPOXIC-ISCHEMIC ENCEPHALOPATHY

Ashley Pak, Elizabeth Lyden, Eric S. Peeples
Creighton University School of Medicine; University of Nebraska Medical Center; Omaha, NE

Background: Hypoxic-ischemic encephalopathy (HIE) can have lifelong detrimental effects including intellectual deficits, epilepsy, sensory loss such as blindness or deafness, or cerebral palsy. After HIE, 40-60% of infants will develop disabilities or die before the age of 2 years. One of the most effective therapies is hypothermia, which can significantly reduce death or lifelong disabilities in patients. In order to optimize the benefits of therapeutic hypothermia, the cooling must begin within the first 6 hours after injury. However, diagnosis currently relies mostly on clinical findings that can be initially subtle, making it difficult to assess the eligibility of the infant for treatment within the therapeutic window. Many studies have sought to identify a single laboratory or clinical diagnostic biomarker in the first hours after injury, but few have attempted to develop prognostic models that integrate both laboratory and clinical values that are typically obtained in the first few hours after injury. By incorporating a mixture of both lab values that have previously shown significance (e.g. nucleated red blood cells [nRBC], lactate, and blood gases) with clinical scores including Apgar and encephalopathy severity, we attempted to develop more representative predictive models to determine those infants most likely to have abnormal brain imaging from HIE.

Significance of Problem: The narrow therapeutic window for initiating therapeutic hypothermia creates limitations in providing timely care for HIE. An effective predictive model could allow for earlier treatment of the highest risk infants, leading to more benefits from therapeutic hypothermia and potentially preventing or attenuating the adversities in neonates with HIE.

Hypothesis, Problem or Question: This study evaluated a combination of laboratory and clinical values available at or around the time of delivery and their effectiveness in prognostic models for predicting abnormal magnetic resonance imaging (MRI).

Experimental Design: This was a retrospective study including infants born between 2012-2022 and treated for HIE with therapeutic hypothermia in the NICUs at Children's Nebraska or Nebraska Medical Center. Patient demographics were collected including gestation age, race, sex, and birth weight. Diagnostic variables that were considered for the model include lab values of nRBC, pH, base deficit, pCO_2, bicarbonate, sodium, creatinine, and lactate as well as clinical values such as encephalopathy severity, intensity of resuscitation, 5-minute Apgar, and 10-minute Apgar scores. The primary outcome was abnormal MRI prior to discharge. MRI findings were abstracted from the clinical neuroradiology interpretation in the chart and reported as the dichotomous value of those with any abnormality versus none. Multivariable logistic regression was used for variables that were significant on univariate analysis to determine predictive value. A backward selection method was used to obtain the best subset of variables to create two predictive models. Model 1 only included variables available immediately upon admission while Model 2 was developed with variables that reflected what a clinician would have available within the first 24 hours after injury.

Results/Data: Data were collected from 139 infants who met the inclusion criteria. Demographic and early outcome data were compared between infants with and without abnormal MRIs. Each of the significant variables in univariate analysis were included in at least one of the two models, unless they had significant collinearity with other variables (e.g. 10-min Apgar with 5-min Apgar, nRBC peak with initial nRBC count, etc). Based on univariate analysis, seven significant variables were initially included in Model 1. Ultimately, after backward selection, the only variable that remained significantly associated with abnormal MRI was the need for chest compressions in delivery room (OR 4.03, $p=0.004$). Overall, Model 1 had an AUC of 0.71. Ten significant variables were initially included in Model 2 and, after backward selection, the need for chest compressions (OR 4.54, $p<0.001$) and peak FiO_2 in the first 24 hours (OR 1.02 for each increase of 0.01 in peak FiO_2, $p= 0.029$) remained. Model 2 had an AUC of 0.68.

Conclusions: The two models provided good and similar prognostic strength to help identify those at risk of abnormal MRI, with model 1 having an AUC of 0.71 and model 2 with an AUC of 0.68. The highest odds in either model was the need for chest compressions. This study suggests that even the prognostic model using only those variables present on admission (Model 1) could allow for early identification of the infants with HIE at highest risk of abnormal MRI and potentially future developmental delays.

THE IMPACT OF LIMB FLEXION ON THE BIOMECHANICS OF THE ARTERIES OF THE CALF IN MEN AND WOMEN

Margarita I. Pipinos[1], Elizabeth A. Amato-Hanner[1], Majid Jadidi[2], Jason N. Mactaggart[1], Alexey V. Kamenskiy[2]

1. University of Nebraska Medical Center - Department of Vascular Surgery, Omaha, NE

2. University of Nebraska Omaha - Department of Biomechanics, Omaha, NE

Introduction: Peripheral Arterial Disease (PAD) operations and reconstructions continue to fail at high rates, particularly below the knee. During limb flexion, distal superficial femoral and popliteal arteries experience significant deformations that are thought to contribute to disease development, but the biomechanics of the tibial arteries below the knee have not been characterized.

Methods: Perfused human cadavers (n=15, 81±9 years old, range 60-93 years, 9F/6M) were imaged with their limbs in standing (180°), walking (110°), sitting (90°), and fetal/gardening (60°) positions using Computerized Tomography (CT). Three-dimensional geometries of the below-knee arteries were reconstructed in Mimics software and used to assess the take-off angle of the anterior tibial artery (AT) and the distance from the AT take-off to the nearest AT side branch.

Results: The AT take-off angle relative to the tibioperoneal trunk increased with more acute limb flexion, from 70±19° in standing to 81±24° in walking, 83±24° in sitting, and 89±27° in fetal/gardening postures ($p<0.01$ for all postures). The AT take-off angle was larger in females than in males (24% in standing and 35% in walking, sitting, and gardening postures, $p<0.01$), but there were no differences between left and right limbs of either sex ($p=0.66$-0.99). The distance from the AT take-off to the nearest AT side branch decreased with more acute limb flexion ($p< 0.01$ for all postures).

Conclusions: Limb flexion increases the AT take-off angle in a sex-dependent manner and compresses the AT, potentially contributing to the differences in hemodynamics and arterial mechanics and affecting the development and progression of tibial artery pathology. A better understanding of the below-knee artery biomechanics can provide valuable insights for surgical planning and may lead to improvements in the success rate and durability of PAD repairs.