50th Annual Scientific Research Symposium

Friday, August 2, 2019
Great Hall
The Mary Duke Biddle Trent Semans Center for Health Education
Αξιος ωφελεϊν τοὺς ἀλγοῦντάς
"Be worthy to serve the suffering"
Priya Kishnani, MD
“Journey from Bench to Bedside and Back: The Clinician Scientist as the Catalyst”
Friday, August 2, 2019
The Mary Duke Biddle Trent Semans Center for Health Education Great Hall

7:30 – 8:00 AM Platform Presentation Setup
Breakfast – Served in the Great Hall Lobby, Level 0

8:00 – 8:30 AM Keynote Address: Priya Kishnani, MD

8:40 – 9:40 AM Platform Presentations

9:45 – 9:55 AM Break

10:00 – 11:00 AM Platform Presentations

11:00 – 12:45 PM Poster Presentations

12:45 – 1:20 PM Pick up lunch – Learning Hall, Level 2
Poster Presenters pick up lunch on 6th Floor

RETURN TO THE GREAT HALL FOR 1:30 PM EVENTS

1:30 – 1:45 PM Lucas Wachsmuth, Davison Council President
New Medical School Class Demographics

1:45 – 2:00 PM Sallie Permar, MD, PhD, Associate Dean
Office of Physician Scientist Development

2:00 – 2:15 PM Dylan Eiger, AOA President
Brief History of Duke Curriculum

2:15 – 2:45 PM Aditee Narayan, M.D., M.P.H, Associate Professor of Pediatrics
Chair, Curriculum Innovations Initiative
Colleen O’Connor Grochowski, Ph.D., Associate Dean for Curricular Affairs

2:45 – 3:00 PM Presentation of Awards
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THE DUKE ΑΩΑ CHAPTER WOULD LIKE TO THANK THE FOLLOWING FOR THEIR PARTICIPATION IN TODAY’S SYMPOSIUM

Platform Judges

Edward Buckley, M.D.
Professor of Ophthalmology; James Pitzer Gills, III, M.D. and Joy Gills Professor of Ophthalmology in the School of Medicine
Professor in Pediatrics
Vice Dean for Education
Chair, Department of Ophthalmology

Daniel Laskowitz, M.D., M.H.S.
Professor and Vice Chair of Neurology Professor of Anesthesiology and Neurobiology
Director, Neurovascular Laboratories

Andrew Alspaugh, MD
Professor of Medicine
Professor in Molecular Genetics and Microbiology

John Matthew Velkey, Ph.D
Assistant Professor of the Practice of Medical Education in the Department of Cell Biology

Poster Judges

Andolsek, Kathryn, M.D., MPH, Associate Director, Graduate Medical Education, Community and Family Medicine
Ashley-Koch, Allison, Ph.D, Professor in Biostatistics and Bioinformatics, Member of Duke Molecular Physiology Institute
Asrani, Sanjay, M.D., Professor of Ophthalmology
Bettger, Janet, ScD, FAHA, Associate Professor of Orthopaedic Surgery Brooks, Megan, M.D., MPH, Assistant Professor of Medicine
Chiba-Falek, Ornit, Ph.D, Professor in Neurology
Cho, Alex, M.D., M.B.A, Assistant Professor of Medicine
Clements, Dennis, M.D., Ph.D, MPH, Professor Emeritus of Pediatrics
Clowse, Megan, M.D., Associate Professor of Medicine
Corsino, Leonor, M.D., Associate Professor of Medicine
Dawson, Geraldine, Ph.D., William Cleland Professor, Director of the Duke Institute for Brain Sciences
Edelman, David, M.D., Professor of Medicine
Engle, Deborah, Ed.D, M.S., Assistant Dean, Assessment and Evaluation
Taylor, Steve, M.D., Adjunct Associate in the Department of Family Medicine and Community Health
Tornai, Martin, Ph.D., Associate Professor of Radiology
Tsalik, Ephraim, M.D., Ph.D., Associate Professor in the Department of Molecular Genetics and Microbiology
Tseng, Henry, M.D., Ph.D., Assistant Professor of Ophthalmology
Welsby, Ian, M.D., Professor of Anesthesiology
Whitson, Heather, M.D., M.H.S., Associate Professor of Medicine
Williams, John, M.D., Professor in Psychiatry and Behavioral Sciences

**Special Thanks**

The Palumbo Family Medical Scholarship is awarded to a School of Medicine graduate who is elected to Alpha Omega Alpha during his or her third year & certified as having financial need by School of Medicine Financial Aid Office. A selection committee chooses the recipient on Medical School Research Day. Selection is based solely on merit, which includes an evaluation of the student's performance during the third year research experience & presentation at Medical School Research Day. The recipient receives a full -tuition scholarship for the fourth year of medical school.

This wonderful scholarship was established by Mr. Arthur Palumbo, a 1949 graduate of Duke University. Mr. Palumbo's brother, the late Dr. Leonard Palumbo, Jr., graduated from Duke Medical School in 1944 & was a distinguished member of our faculty. In addition to establishing the medical scholarship we are awarding today, Mr. Palumbo established the Leonard Palumbo Jr., M.D. Faculty Achievement Award Endowment Fund in memory of his brother. Income from this fund provides support for an annual award to one or more medical school faculty members who has best displayed understanding & dedication to compassionate patient care & excellence in the teaching of young physicians.

Mr. Palumbo is also a major benefactor of Duke Children's Hospital- the T level of our McGovern Davison Children's Health Center has been renamed the Arena - Palumbo Research & Education Center in honor of Mr. Palumbo & former Duke Pediatrician Jay Arena- a friend of Mr. Palumbo whom he greatly admired.

Mr. Palumbo passed away on June 20th at the age of 91. He was a wonderful man whose extraordinary philanthropy has touched the lives of medical students, faculty, and countless patients.
PLATFORM PRESENTATIONS
8:40-8:50 – Nathan Brajer
*Prospective and External Evaluation of a Machine Learning Model to Predict In-Hospital Mortality*
Mentor: Adrian Hernandez, M.D.
Study Program: MBA Dual Degree; Jennifer Perkins, MD, MBA Director

8:50-9:00 – Alexandra Bocharnikov
*Expanded T peripheral helper cells promote B cells responses in systemic lupus erythematosus via IL-21 and transcription factor MAF*
Mentor: Deepak Rao, M.D., PhD.
Study Program: Primary Care Leadership Track; Anh Tran, PhD, MPH, Director

9:00-9:10 – Chelsea Handfield
*Activation of nociceptive fibers following skin injury triggers antiviral host defense immunity*
Mentor: Amanda MacLeod, M.D.
Study Program: Microbiology, Immunology and Infectious Disease; Andrew Alspaugh, MD, Director
Poindexter Fellowship (2017-2018) and Stead Fellowship (2018-2019)

9:10-9:20 – Vinay Giri
*Feasibility of Home-Based Hematopoietic Stem Cell Transplantation*
Mentor: Anthony Sung, M.D.
Study Program: Clinical Research; Vivian Chu, MD, Director
Eugene A. Stead Research Scholarship, ASH HONORS Award

9:20-9:30 – Thomas Neufeld
*In Vivo and In Vitro Metabolic Profiling of ANGPTL3 Deficiency*
Mentor: Nathan Stitziel, M.D., Ph.D
Study Program: Cardiovascular; Neil Freedman, M.D., Director
Sarnoff Cardiovascular Research Fellowship

9:30-9:40 – Rebecca Vernon
*Vascularized Composite Allotransplantation: A functional hind limb model in mice*
Mentor: Linda Cendales, M.D.
Study Program: Anesthesiology, Surgery, and Environmental Physiology; Richard Moon, M.D., Director
Duke Health Scholars Award (LC)

9:45-9:55 – Break
10:00-10:10 – Ouwen Huang
Mimicking Clinical Image Post-Processing with Deep Learning
Mentor: Mark Palmeri, M.D., Ph.D.
Study Program: Medical Scientist Training Program
National Institute of Biomedical Imaging and Bioengineering under Grant R01-EB026574.

10:10-10:20 – Cosette Champion
Lymphopenia and bone marrow T-cell sequestration accompanying stroke are mediated by T-cell S1P1 loss
Mentor: Peter Fecci, M.D., Ph.D.
Study Program: Neuroscience; Peter Fecci, M.D., Ph.D., Director

10:20-10:30 – Ravyn Njagu
Impact of the Healthy Outcomes in Pregnancy with SLE through Education of Providers (HOP-STEP) Program: A Mixed Methods Approach
Mentor: Megan E.B. Clowse, M.D.
Study Program: Clinical Research; Vivian Chu, MD, Director
GlaxoSmithKline; Rheumatology Research Foundation Preceptorship

10:30-10:40 – John Tanaka
Anaerobic Antibiotics and the Risk of Graft-Versus-Host Disease after Allogeneic Hematopoietic Stem Cell Transplantation
Mentor: Matthew Kelly, M.D., MPH
Study Program: Microbiology, Immunology and Infectious Disease; Andrew Alspaugh, MD, Director
IDSA Medical Scholars Program

10:40-10:50 – Victoria Wickenheisser
Light Mediated Therapeutics in Arthritis
Mentor: Teresa Tarrant, M.D.
Study Program: Microbiology, Immunology and Infectious Disease; Andrew Alspaugh, MD, Director
Eugene A. Stead Student Research Scholarship; Rheumatology Research Foundation (RRF) Medical Student Preceptorship; RRF Innovative Research Award

10:50-11:00 – Karishma Sriram
How Much is Enough? The association between physical activity and cardiometabolic health in adolescents
Mentor: Charlene Wong, M.D.
Study Program: Clinical Research; Vivian Chu, MD, Director
Prospective and External Evaluation of a Machine Learning Model to Predict In-Hospital Mortality

Nathan Brajer, Brian Cozzi, Michael Gao, Marshall Nichols, Mike Revoir, Suresh Balu, Joseph Futoma, Jonathan Bae, Noppon Setji, Adrian Hernandez, Mark Sendak

Background: The ability to accurately predict in-hospital mortality for patients at the time of admission could improve clinical and operational decision-making and outcomes. Few machine learning models have been developed to predict in-hospital death that are both broadly applicable to all adult patients across a health system and readily implementable, and, to the best of our knowledge, none have been implemented, evaluated prospectively, or externally validated. The primary objective of this study was to prospectively and externally validate a machine learning model that predicts in-hospital mortality for all adult patients at the time of hospital admission. Secondary objectives were to design the model using commonly available EHR data and accessible computational methods.

Methods: In a retrospective cohort study, electronic health record (EHR) data from a total of 43,180 hospitalizations representing 31,003 unique adult patients admitted to a quaternary academic hospital (Hospital A) from October 1, 2014 to December 31, 2015 formed a training and validation cohort. We utilized a Gradient Boosting model using the XGBoost package in Python. Model performance for predicting an outcome of in-hospital mortality was quantified using the area under the receiver operating characteristic curve (AUROC) and area under the precision recall curve (AUPRC). The model was further validated in additional retrospective cohort studies spanning from March 1, 2018 to August 31, 2018, using 16,122 hospitalizations representing 13,094 unique adult patients admitted to Hospital A, 6,586 hospitalizations representing 5,613 unique adult patients admitted to Hospital B, and 4,086 hospitalizations representing 3,428 unique adult patients admitted to Hospital C. Lastly, the model was integrated into the production EHR system and prospectively validated on a cohort of 5,273 hospitalizations representing 4,525 unique adult patients admitted to Hospital A between February 14, 2019 and April 15, 2019. To better understand how the model should be used in the hospital setting, we partnered with clinical leaders to iteratively developed a workflow decision framework and a simple user interface showing model output using Apache Superset. Lastly, we developed a model facts sheet, similar to an over-the-counter drug label, that clearly communicates important information about the model to clinician end users.

Results: A total of 75,247 hospital admissions (median [IQR] age 59.5 [29.0] years; male [45.9%]) were included in the study. The in-hospital mortality rates for the training validation, retrospective validations at Hospitals A, B, and C, and prospective validation cohorts, respectively, were 3.0%, 2.7%, 1.8%, 2.1%, and 1.6%. The area under the receiver operating characteristic curves (AUROCs), respectively, were 0.87 (0.83-0.89), 0.85 (0.83-0.87), 0.89 (0.86 – 0.92), 0.84 (0.80-0.89), and 0.86 (0.83-0.90). The area under the precision recall curves (AUPRCs), respectively, were 0.29 (0.25-0.37), 0.17 (0.13-0.22), 0.22 (0.14-0.31), 0.13 (0.08-0.21), and 0.14 (0.09-0.21).

Conclusion: The results demonstrated accurate prediction of in-hospital mortality for adult patients at the time of admission. The data elements, methods, and patient selection make the model implementable at a system-level. The user interface, workflow decision framework, and model facts sheet supported efforts to develop workflows utilizing model output. We plan to evaluate the impact on operational and clinical outcomes in future studies.
Expanded T peripheral helper cells promote B cells responses in systemic lupus erythematosus via IL-21 and transcription factor MAF

Alexandra V. Bocharnikov, Vanessa S. Wacleche, Ye Cao, Deepak A. Rao
Rheumatology Research Foundation Mentorship Award & Science Development Award

Background: Pathologic T cell-B cell interactions are hallmark features of many autoimmune diseases. CXCR5+ PD-1+ T follicular helper (Tfh) cells are generally considered the principal T cell population capable of helping B cells and driving auto-antibody responses in the lymphoid follicles, yet distinct T cell populations can augment B cell responses within chronically inflamed peripheral tissues. PD-1hi CXCR5- T peripheral helper (Tph) cells are B cell-helper T cells that are highly expanded in the circulation of SLE patients that have been found to promote plasmablast and antibody production in B cells in the periphery. However, the mechanism of how Tph cells provide B cell help is unknown. While Tfh cells depend on the transcription factor Bcl6 to produce IL-21, a cytokine that induces plasmablast production, Tph cells do not express high levels of Bcl6. This suggests that transcription factors other than Bcl6 may control B cell-helper function in human CD4+ T cells and could serve as targets for controlling SLE disease.

Methods: MAF expression and expansion of human SLE CD4+ T cell subsets was determined by low-input RNA seq RNA-seq and flow cytometry. To evaluate the role of MAF, we disrupted the gene encoding MAF by nucleofection of a CRISPR/Cas9 ribonucleoprotein (RNP) complex in primary CD4+ T cells and in sorted Tph cells. Expression of IL-21 and other cytokines was determined by RT-PCR and ELISA. B cell-helper function of CRISPR-treated T cells was evaluated by co-culturing sorted T cell subsets from SLE or control donors with memory B cells plus SEB. Plasmablast formation was determined by flow cytometry.

Results: RNA-seq analyses of CD4+ T cell subsets revealed high expression of MAF, but not BCL6, in both Tph cells and Tfh cells sorted from blood of RA and SLE patients. After 2 days of CD3/CD28 stimulation, Tph cells and Tfh cells expressed >2-fold higher levels of MAF protein than did naive or PD-1neg memory CD4+ T cells. High expression of MAF in both Tph cells and Tfh cells, two subsets that produce IL-21, suggested a possible connection between MAF and IL-21. To test this, we deleted MAF in primary human CD4+ T cells by nucleofection of a MAF-targeting CRISPR/Cas9 RNP. This method abrogated MAF protein expression in 80% of T cells. Compared to a control CRISPR targeting CD8, MAF deletion reduced mRNA expression of IL21 and IL10 in 4 independent donors and inhibited secretion of IL-21 protein. Using Tph cells sorted from blood of SLE patients, we confirmed that Tph cells stimulate differentiation of memory B cells into plasmablasts in an IL-21-dependent manner, as neutralization of IL-21, but not IL-10, inhibited plasmablast formation by ~50%. To evaluate the effect of MAF on B cell-helper function, we deleted MAF by CRISPR in sorted human Tph cells from SLE patients or controls and co-cultured these cells with memory B cells. Deleting MAF reduced the ability of Tph cells to induce B cell differentiation into plasmablasts by 50% compared Tph cells treated with a control RNP.

Conclusion: High expression of MAF is a common feature of both Tph cells and Tfh cells, two T cell populations that produce high levels of IL-21. Loss of MAF inhibits IL-21 expression in primary human CD4+ T cells and abrogates the ability of Tph cells to help B cells. MAF may regulate key components of T cell help to B cells in autoimmune conditions, specifically in SLE. Defining the T cell populations in SLE and the mechanisms in which they drive B cell responses, such as MAF, may enable design of therapies that specifically target disease promoting cell subsets and the development of pathologic antibodies.
Activation of nociceptive fibers following skin injury triggers antiviral host defense immunity

Chelsea Handfield, Jeffery Kwock, Qingjian Han, Kaiyuan Wang, Ru-Rong Ji, Amanda S. MacLeod
Poindexter Fellowship (2017-2018) and Stead Fellowship (2018-2019)

Background: Multiple innate immune signaling pathways become activated upon skin injury in order to reestablish the antimicrobial barrier, prevent infection and excessive tissue injury. Damage to the skin barrier often elicits pain and leads to activation of cutaneous nociceptive fibers. We hypothesized that nociceptors in the skin are activated upon breach of the skin barrier and contribute to the innate antiviral response to skin injury. Calcitonin gene related peptide (CGRP) is a neuropeptide released by TRP+ nerve fibers upon activation. It binds to the CALCRL receptor which can be expressed by CD301+ dendritic cells. The neuromediator N-acetyl-galactosamine (GalNAc) is present in sensory nerves and binds with high specificity to the CD301 lectin. CD301 is a marker for the subset of dendritic cells that release IL-27 in the process of skin wound repair. Our lab has demonstrated that IL-27 is a novel regulator of antiviral proteins.

Methods: WT, RTX-treated and Trpv1−/− mice all underwent excisional wounds in the morning. Non-wounded and wounded skin was analyzed by qPCR for gene transcription and IF for protein expression. Immature dendritic cells (iDCs) were derived from human THP1 monocytes using rhIL-4 and rhGM-CSF. We obtained fresh human skin from plastic surgery patients undergoing abdominoplasty. Epidermis was separated from dermis using collagenase and dispase. We then took the epidermal cells and FACs sorted for Langerhans cells (LCs). iDCs and LCs were stimulated with GalNAc 200uM for 3hrs and analyzed by qPCR. All data was analyzed via student’s T-test and represented as mean +/- SEM.

Results: Resiniferatoxin (RTX)-treated mice showed preferential ablation of Trpv1+ nerve fibers in the skin. Our excisional wound assays demonstrated that RTX pharmacologic ablation of Trpv1 channels reduced the transcription of antiviral proteins Oas2 (p<0.01), Oasl2 (p<0.01), Isg15 (p<0.001) and Mx1 (p<0.05) in the skin upon wounding. RTX treated mice also show a decrease in the expression of Oas2 within the skin at the wound edge by IF. We confirmed these findings using Trpv1−/− mice who again showed a decrease in the transcription of antiviral proteins Oas2 (p<0.05) and Oasl2 (p<0.05) upon wounding and a reduction in the expression of Oas2 within wounded skin. Further, intradermal injections of CGRP into normal murine skin increased the transcription of Oas2 (p<0.001). iDCs and LCs isolated from fresh human skin express IL27 when stimulated with GalNAc (p<0.01 and p<0.001, respectively). Given the knowledge from previous work that cathelicidin LL37 is produced within the skin following injury and complexes with nucleic acids to enhance production of type I IFNs, we tested the possibility that LL-37 could also alter GalNAc signaling. We demonstrate that LL37 enhances binding of GalNAc polymers to iDCs and that LL37 given in combination with GalNAc significantly enhances transcription of IL27 (p<0.001), IFNa4 (p<0.01), and IFNb1 (p<0.01).

Conclusions: Here, we show that TRPV1 signaling is necessary and sufficient for the production of antiviral proteins following acute skin injury. Additionally, the neuromediator GalNAc is capable of inducing antiviral upstream mediators IL-27 and type I IFNs. Together, our data suggest that noiception promotes skin antiviral competence through activation of antiviral signaling pathways upon wounding. Better understanding of the role of pain and itch in stimulating antimicrobial peptide and protein expression and induction following injury is relevant for improving skin repair.
Feasibility of Home-Based Hematopoietic Stem Cell Transplantation

Vinay K. Giri, Kristi Romero, Tara Dalton, MS, Rebecca Shelby, PhD, Krista R. Nichols, MSN, Anthony D. Sung, MD, Nelson J. Chao, MD

Eugene A. Stead Research Scholarship, ASH HONORS Award

Background: Roughly 20,000 individuals undergo potentially life-saving allogeneic (allo) and autologous (auto) hematopoietic stem cell transplantation (HSCT) in the United States every year to treat a variety of malignant and non-malignant conditions. These patients require extensive hospitalizations or daily clinic visits for the duration of their transplant, increasing the risk of nosocomial infection or gut microbiome disruption, which in the case of allo patients might lead to the development of graft versus host disease (GVHD). By keeping patients at home during their transplant, as previously done at the Karolinska Institute in Sweden, we may enhance patient quality of life, preserve the gut microbiome, and improve outcomes.

Methods: We conducted a phase 1 study of home-based adult HSCT to investigate the feasibility and safety of implementing this strategy in the United States. Study participants received pre-transplant conditioning and stem cell infusion at the hospital or clinic, but were then discharged home for the remainder of their transplant course. APPs made daily house calls to examine patients, draw blood for laboratory studies, and administer fluids or provide blood transfusions as needed. Patients would return to the clinic or hospital to manage acute events. Quality of life was assessed via the FACT-BMT through the transplant course. Stool samples were collected at baseline and the first four weeks after transplant and underwent 16s rRNA sequencing for taxonomic identification of gut microbial composition. Clinical outcomes were tracked for one year post-transplant. Upon completion of the study, each home transplant patient was matched with two controls who had previously received standard of care treatment based on demographic factors such as age, disease, and type of transplant.

Results: Twenty-five patients received home HSCT, including 8 allos and 17 autos. On average, allo patients spent 66% of their days entirely at home, and auto patients remained home for 58% of their days. Outcomes such as bloodstream infection, C. diff infection, CMV reactivation, febrile neutropenia, relapse, treatment-related mortality, and acute and chronic GVHD were not found to be significantly different between the home transplant patients and their matched controls. Four home patients and five of their matched controls had stool samples available for analysis of gut microbial composition before and after transplant. Home patients experienced a milder decrease in mean alpha diversity (ΔH -0.136) compared to their matched controls (ΔH -0.212), though there were not enough samples to power a statistical test of this comparison. Quality of life was well-preserved for all home patients as assessed by the FACT-BMT with autos in particular demonstrating an increase in mean quality of life score from baseline to Day 100 (+11.6, p=0.031). Patients and caregivers endorsed the program, providing numerous expressions of gratitude on exit interviews.

Conclusions: These results suggest that home transplant is safe and feasible. Home-based patients were able to maintain their quality of life and had comparable outcomes to their matched controls. While most patients did return periodically to the hospital or clinic, keeping patients out of the hospital for even half the duration of transplant might lead to substantial cost savings. Microbiome analysis suggests a trend to better preservation of pre-transplant gut microbiota, and as additional samples from this study are processed, further investigation of diversity and intestinal domination will be conducted. A phase 2 randomized trial of home vs standard transplant is currently underway to better compare outcomes and costs.
In vivo and in vitro Metabolic profiling of ANGPTL3 deficiency

Thomas Neufeld, Santhi Pondugula, Tyler Fraum, Katie Fowler, Michele Di Martino, Marcello Arca, Nathan Stitziel

Funding: Sarnoff Cardiovascular Research Fellowship

Background: ANGPTL3 is a hepatocyte-derived inhibitor of lipoprotein lipase (LPL). ANGPTL3 deficiency in humans confers a phenotype known as familial combined hypolipidemia (FHBL2), characterized by low circulating levels of low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglyceride rich lipoproteins (TRLs). The mechanism of LDL-C reduction in ANGPTL3 deficiency is incompletely understood. Clinical phenotyping of ANGPTL3-mutant subjects and in vitro studies using iPSC-derived hepatocyte-like cells (HLCs) were performed to further understand how ANGPTL3 deficiency reduces plasma LDL-C and modifies atherosclerotic disease risk.

Methods: Clinical phenotyping was performed on human subjects in Rome and St. Louis. 6 ANGPTL3-mutant homozygotes (ANGPTL3-KO), 28 heterozygotes, and 60 related controls (WT), underwent clinical phenotyping. Hepatic fat quantification by MRI and MRS, coronary CT angiography, and kinetic studies of lipoprotein metabolism were performed.

In Vitro Studies: Peripheral blood mononuclear cells (PBMCs) were collected from 2 ANGPTL3-KO and 2 WT subjects, and reprogrammed to iPSCs via Sendai virus transfection of Yamanaka factor transgene constructs. iPSCs were differentiated into HLCs using an established growth factor-based protocol. qPCR was performed to investigate transcriptional changes related to lipid and glucose metabolism. Hepatic lipoprotein synthesis was investigated by quantifying ApoB production and secretion in mutant and control cells. Nascent intracellular ApoB was labeled using the methionine analog AHA (L- Azidohomoalanine). ApoB secretion was quantified by immunoprecipitation of ApoB from the media of mature HLCs.

Results: Mean hepatic fat fraction was not significantly different according to ANGPTL3 genotype in either the Italian (4.24% in homozygotes, 6.2% in heterozygotes, and 7.7% in wild type) or St. Louis (21% in homozygotes, 25.6% in WT) cohorts. In vivo lipoprotein kinetics demonstrated that complete ANGPTL3 deficiency decreased very low-density lipoprotein (VLDL) production rate (8.6 vs 26.9 mg/kg/day) and increased LDL fractional turnover rate (0.052 vs 0.027 pools/h). Coronary CT angiogram and MRS data are currently being analyzed.

In Vitro Studies: qPCR performed on ANGPTL3-KO HLCs demonstrated significant changes in lipid trafficking transcripts compared to WT HLCs, including a 1.8-fold increase in LDL Receptor (LDLR), a 2.3-fold increase in hepatic triglyceride lipase (HTL), and a 2.8-fold reduction in phospholipid transfer protein (PLTP). Gluconeogenic genes including PGC1α (2.6-fold) and PCK (2-fold) were increased relative to WT HLCs. ANGPTL3-KO HLCs produced less ApoB over the course of 4 hours than WT HLCs, while no difference in ApoB secretion was observed.

Conclusions: HLCs from ANGPTL3-KO subjects demonstrate reduced production of ApoB, as well as transcriptional changes consistent with decreased intrahepatic VLDL lipidation and increased lipoprotein clearance from the plasma. These findings correlate with the kinetic and radiologic studies performed on ANGPTL3 patient cohorts. Further study will include directly testing lipid and glucose uptake by ANGPTL3-KO HLCs, as well as attempting to rescue the phenotype of ANGPTL3 deficiency using CRISPR-Cas9 technology.
Vascularized Composite Allotransplantation: A Functional Hind Limb Model in Mice

Rebecca Vernon, Jun Wang, Mingqing Song, Natasha Wilson, Linda Cendales

*Duke Health Scholars Award* (LC)

**Background:** Vascularized composite allograft (VCA) has emerged as a potential reconstructive therapy for patients who have suffered severe tissue loss. Animal models are critical for understanding the unique mechanisms of rejection in VCA. Although the mouse is considered the gold standard in transplantation research, it remains a challenging model system. We present a functional mouse model of orthotopic hind limb transplantation using end-to-side anastomoses of the donor aorta and inferior vena cava (IVC) to the respective recipient vessels (Figure 1). To the best of our knowledge, this approach has not been reported in the scientific literature.

**Methods:** All transplants were performed by a single surgeon. A total of 13 syngeneic (C57BL/6 to C57BL/6) and 10 fully mismatched allogeneic (C3H to C57BL/6) transplants were performed. Skin samples from the grafts were collected at the time of euthanasia.

**Results:** The overall technical success rate of the syngeneic grafts was 54% (7 out of 13). Five syngeneic mice survived for over 90 days after transplant. Syngeneic grafts did not exhibit either clinical or histological signs of rejection. At the time of euthanasia, these mice exhibited near-normal hair growth and had regained some motor function, including the ability to rear on the hind legs. The technical success rate of the allogeneic transplants was 100% (10 out of 10). Nine allografts displayed clinical signs of acute rejection by 9-10 days post-operatively. One allograft recipient did not exhibit clinical signs of rejection until post-op day 15. Histological examination of the allograft skin at the time of euthanasia revealed acute rejection (Banff III). The overall technical success rate of all transplants described in this study was 74% (17 out of 23).

**Conclusions:** The mouse remains a challenging model system for hind limb transplantation due to its small size, low blood volume, and physiologic fragility compared to rats and large animals. We demonstrate the feasibility of orthotopic hind limb transplantation using end-to-side anastomoses of the donor aorta and IVC to the respective recipient vessels in a mouse model. Superior technical outcomes in the allogeneic cohort were most likely due to increased experience with this technique over time. This approach avoids the challenge of completing submillimeter end-to-end anastomoses of the femoral vessels and can achieve long-term survival outcomes, with syngeneic transplant recipients surviving over 90 days postoperatively.

*Figure 1.* End-to-side anastomosis of the donor aorta to the recipient aorta in an orthotopic hind limb transplant in mouse.
Mimicking Clinical Image Post-Processing with Deep Learning

Ouwen Huang, Willie Long, Dr. Sina Farsiu, Dr. Gregg Trahey, Dr. Mark Palmeri
National Institute of Biomedical Imaging and Bioengineering under Grant R01- EB026574. The authors would like to thank Siemens Medical Inc. USA.

Background, Motivation and Objective: Ultrasound post-processing is used in most diagnostic-grade scanners to dramatically improve overall image quality (e.g. reduce speckle noise, enhance contrast). These post-processing techniques vary across manufacturers and are generally kept secret, presenting difficulties for researchers looking to fairly benchmark against current clinical imaging baselines. We provide a deep learning method that closely replicates post-processing on a commercial scanner using “as is” images produced by the scanner and unpaired delay-and-summed (DAS) beamformed IQ data from a research scanner. Benchmarks of the replica algorithm show it can run in real-time for practical imaging purposes.

Methods: We collect 39200 ultrasound IQ data still frames using a Siemens S2000 and Verasonics scanner; a compiled post-processing software is applied to each image creating an IQ and post-processed pair. Paired images are randomly train/test split to 30000/9200; the images are unpaired, independently shuffled, and randomly cropped to 512x512. We train a cyclical generative adversarial network (CycleGAN) to map our IQ dataset to resemble post-processed data. We use student-teacher training to simplify our CycleGAN generator from 7.2M to 35k parameters. We compare our results with the original proprietary post-processed images, and a simple U-net trained on paired images.

Results/Discussion: Our CycleGAN student model achieves a mean absolute error (MAE) of 3.65% per pixel. On average, the model processes 512x512, 12-bit IQ data at 92Hz—enough for real-time applications. Previous work has used GANs to estimate speckle reduction using paired images. We show that if paired images are available, a non-GAN U-net can achieve similar results. Additionally, we implement a CycleGAN to estimate commercial post-processing, which performs non-linear processing for contrast enhancement and hole filling in addition to speckle reduction, using only unpaired images. Our results show it is feasible to replicate any commercial post-processing by taking images from a commercial scanner, collecting raw data from similar targets using a separate system, and applying our method. We plan to provide our models open source to the research community as a useful diagnostic imaging comparison tool.

![Image of results](image.png)

**Figure 1.** Zoomed in images of fetus (top left), liver (top right), phantom (bottom left). Our Student CycleGAN estimate is qualitatively similar to diagnostic post-processing. We discern some subtle differences in contrast between images. In the phantom, we note a bright scatterer which is suppressed by diagnostic post-processing, but kept by our methods which could account for the 3-6% absolute error deviation. The table (bottom right) contains quantitative metrics on absolute error against the diagnostic post-processing image.
Lymphopenia and bone marrow T-cell sequestration accompanying stroke are mediated by T-cell S1P1 loss

Cosette Champion, Pakawat Chongsathidkiet MD, Daniel Wilkinson PhD, Haichen Wang MD, Xiuyu Cui MS, Daniel Laskowitz MD, Peter Fecci MD PhD

Background: Stroke is a major cause of death and disability in the United States. Stroke leads to systemic immunosuppression (stroke-induced immunodepression, or SIID), including lymphopenia and lymphoid organ shrinkage, but the mechanisms governing this are incompletely understood. Ultimately, this immunosuppression contributes to infections, which occur in ~30% of patients following stroke, and contribute significant morbidity. Our lab recently characterized the novel phenomenon of bone marrow T-cell sequestration in the setting of intracranial tumors. T-cells missing from the blood and lymphoid organs instead accumulate in the bone marrow in a process mediated by loss of the sphingosine-1-phosphate receptor 1 (S1P1) from the T-cell surface. This phenomenon occurs in models of glioblastoma as well as intracranial melanoma, lung and breast cancers, suggesting a common CNS mechanism rather than a tumor-specific phenomenon. We hypothesized that the same phenomenon may occur in other types of CNS injury, such as stroke.

Methods: Ischemic stroke in mice was modeled using transient middle cerebral artery occlusion (MCAO) surgery. For control groups, sham surgery was performed by making a midline incision and exposing but not occluding the artery. Blood, bone marrow, and spleens were collected from mice at day 2, 5, 7 or 14 following stroke or sham surgery and tissues analyzed by flow cytometry. T-cell surface S1P1 levels were assessed, as were T-cell counts in each compartment.

Results: Transient decreases in blood CD4+ and CD8+ T-cells, and splenic involution occurred following MCAO, while sham surgery resulted in transient increase in blood T cell counts. CD4+ and CD8+ T-cells accumulated in the bone marrow of mice after MCAO, with CD4+ T-cell counts increasing 2-fold from baseline by day 7, and CD8+ T-cell counts increasing 3-fold. No such changes occurred following sham surgery. T-cell numbers peaked at day 7 post-MCAO before returning to normal by day 14. Bone marrow T-cells were predominantly naïve (CD62LhiCD44lo) rather than memory (CD44hi). Bone marrow T-cells after MCAO demonstrated decreased levels of S1P1 on their surface compared to T-cells in the marrow of mice after sham surgery.

Conclusions: Bone marrow T-cell sequestration occurs transiently following stroke and is mediated by the S1P-S1P1 axis. This may prove to be an adaptive mechanism to limit intracranial inflammation following an initial insult. Better understanding of this phenomenon may shed further light on a novel mechanism of immune privilege and allow for therapeutic modulation in the setting of stroke, brain tumor, and other types of intracranial injury.
Impact of the Healthy Outcomes in Pregnancy with SLE through Education of Providers (HOP-STEP) Program: A Mixed Methods Approach

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GlaxoSmithKline; Rheumatology Research Foundation Preceptorship

Background: While centers have worked to optimize lupus pregnancy outcomes, additional efforts are required to have a broader impact on lupus pregnancy outcomes. The goal of this educational initiative was to create a multi-dimensional intervention to equip community rheumatologists with the needed skills, attitudes, and confidence to effectively manage contraceptive decisions and improve pregnancy outcomes.

Methods: Design of the HOP-STEP program included a needs assessment followed by development of a comprehensive program using mixed modalities. The program included an in-person didactic, a simulated clinical experience, training in use of a comprehensive handout to guide open contraception and pregnancy conversations, and access to an innovative website. A survey was emailed to 149 individuals before and after workshop completion. All attendees were invited to be interviewed about their experience integrating HOP-STEP resources into practice.

Results: We analyzed 68 pre-surveys and 55 post-surveys. For qualitative analysis, 8 interviews were completed until thematic saturation was achieved. At program completion, there was an increase number of providers who have a systematic approach to preparing a woman with lupus for pregnancy, 45.6% to 94.6%; p<0.0001. When assessed using confidence scale (0=no confidence, 100=high confidence), median provider confidence in helping women with lupus choose appropriate contraception increased from 59 to 89; p<0.0001 and confidence in choosing pregnancy compatible medications increased from 66 to 91; p<0.0001. While participants demonstrated limited change in contraceptive knowledge, an emerging theme was use of the HOP-STEP contraception handout:

“I’m not really comfortable recommending birth control method[s], but it was interesting to see that whole [contraception] chart…it did increase my confidence because I could just cheat and look at it and show [patients] visually.”

After the program more providers correctly identified azathioprine (74% to 98%, p <0.0001) and tacrolimus (46% to 91%, p <0.0001) as pregnancy compatible and mycophenolate as teratogenic (84% to 96%, p=0.04). Regarding comparative teratogenicity, a common theme was elucidated by the following quote:

“That Cellcept is as high as it is on the list of drugs that are very bad for fetuses…I didn’t realize that quite as much as I should’ve, everybody knows about methotrexate, but the fact that Cellcept is probably even worse than methotrexate I was not aware of...”

Conclusion: We have demonstrated successful creation and delivery of a new multi-modal educational program, HOP-STEP, that has improved provider confidence, skills, and knowledge in managing women with lupus who desire pregnancy. Providers may now access a unique curriculum and resources that encourage providers and patients to have open and accurate conversations about pregnancy, creating lasting clinical change.
Anaerobic Antibiotics and the Risk of Graft-Versus-Host Disease after Allogeneic Hematopoietic Stem Cell Transplantation

John S. Tanaka, BA, Rebecca R. Young, MS, Matthew S. Kelly, MD, MPH
IDSA Medical Scholars Program

Background: Bacteria within the gut interact extensively with the host immune system and thus may modify the risk of graft-versus-host disease (GVHD) after allogeneic hematopoietic stem cell transplantation (HSCT). During the post-transplant neutropenic period, 80-86% of allogeneic HSCT recipients experience at least one febrile episode, and current guidelines recommend empirical therapy with broad-spectrum antibiotics at first sign of febrile neutropenia. Although each of these antibiotics can have a substantial impact on the gut microbiota, only piperacillin-tazobactam and carbapenems have appreciable activity against anaerobic bacteria. Recent studies suggest that commensal anaerobic gut bacteria play an important role in gut homeostasis and may be protect from the development of GVHD. We hypothesized that receipt of an antibiotic regimen with an anaerobic spectrum of activity is associated with a higher risk of grade II-IV acute GVHD when compared to non-anaerobic antibiotic regimens.

Methods: In this single-center retrospective cohort study, we evaluated associations between peri-transplant receipt of antibiotics with an anaerobic spectrum of activity and the risk and severity of GVHD among 877 adult allogeneic HSCT recipients. We identified patients who developed febrile neutropenia after HSCT and compared GVHD risk and mortality among patients who received anaerobic antibiotics (piperacillin-tazobactam or carbapenems; n=333) to patients who received only antibiotics with minimal activity against anaerobes (aztreonam, ceftazidime, or cefepime; n=276). Antibiotics received by patients between 7 days before and 28 days after allogeneic HSCT and GVHD diagnoses were verified via manual review of medication orders and provider notes in electronic medical records.

Results: We found that receipt of anaerobic antibiotics was associated with increased risks of grade II-IV acute GVHD (hazard ratio (HR): 1.41; 95% confidence interval (CI): 1.10–1.79; P=0.01) and acute GVHD mortality (HR: 1.87; 95% CI: 1.13, 3.11; P=0.02). This association was driven primarily by an association with acute GVHD of the gut or liver only (HR: 1.71; 95% CI: 1.09, 2.67; P=0.02) and was observed with even short (<7 days) courses of anaerobic antibiotics. Anaerobic antibiotic exposure was not associated with acute skin GVHD (HR: 0.97; 95% CI: 0.69, 1.37; P=0.88), chronic GVHD diagnosis (HR: 0.93; 95% CI: 0.70, 1.23; P=0.43), or chronic GVHD mortality (HR: 0.89; 95% CI: 0.44, 1.81; P=0.76).

Conclusions: Receipt of anaerobic antibiotics for febrile neutropenia is associated with an increased risks of acute gut or liver GVHD and acute GVHD mortality. Our results suggest that limiting use of antibiotics with an anaerobic spectrum of activity after allogeneic HSCT may reduce acute GVHD incidence and mortality.
Light Mediated Therapeutics in Arthritis

Victoria Wickenheisser, Emily Rabjohns, Emilia Zywot, Natalia Oriola, Christina Marvin, Song Ding, David Lawrence, Teresa Tarrant

Eugene A. Stead Student Research Scholarship; Rheumatology Research Foundation (RRF) Medical Student Preceptorship; RRF Innovative Research Award

Background: Rheumatoid arthritis (RA) therapies are constrained by the failure to deliver sufficient quantities of drug to the inflamed site, systemic side effects, and the inability for the patient to self-direct therapeutics in a site-targeted fashion. To address this, we have conjugated anti-inflammatory drugs to the light-responsive fluorophore Cy5, anchored to vitamin B12. These photocleavable drug-conjugates can be loaded into red blood cells (RBCs) and released only after laser light activation. In vitro studies have shown RBC-B12-Cy5-dexamethasone successfully induced nuclear migration of the glucocorticoid receptor after laser light photocleavage. We believe this system can be used in the treatment of RA, where externally and selectively applied laser to joints can trigger photocleavage of anti-inflammatories internally loaded into RBCs to the targeted site.

Methods: Collagen Antibody Induced Arthritis (CAIA) was induced in 45 DBA1J mice. Arthritis was measured by a blinded observer with a clinical disease score index, and mice were randomized to 3 groups after symptom onset: RBC-B12-Cy5-dexamethasone (RBC-DEX), RBC-Cy5 (negative control), intraperitoneal dexamethasone (IP-DEX; positive control). Hypotonic solution was used to create a porous membrane in murine RBCs for drug uptake, followed by isotonic solution to close the pores and trap the phototherapeutic inside until photo-release. The RBC-DEX group received RBCs (90% hematocrit, 100μL) loaded with B12-Cy5-dexamethasone (approx. 0.0065 mg) intravenously. The RBC-Cy5 control group received RBCs internally loaded with only B12-Cy5. Intravenous injections were given one time at symptom onset for both RBC-DEX and RBC-Cy5. The IP-DEX group received 0.5mg/kg daily until a clinical score of 0 in the arthritic paw receiving laser. Laser (635 nm, 3 mW) was applied to one affected joint for 5 minutes immediately following i.v. or i.p. injection (based on group assignment) and each day until termination. There were no adverse reactions from laser application.

Results: RBC-DEX and IP-DEX produce significant improvement in clinical arthritis compared to the control RBC-Cy5 (p=0.0007, p=0.0002 respectively), but do not significantly differ from each other (p=0.6) (Fig. 1). The RBC-DEX group received on average 80% less dexamethasone as compared to the IP-DEX treatment group, without significantly different results.

Conclusion: RBC-DEX is an effective CAIA treatment compared to negative control and is as effective as the positive drug control using a substantially lower dose of dexamethasone. This warrants further study into the parameters that are required for selective release of RBC-DEX in arthritis treatment.

Figure 1. Assessment of mean arthritis severity score after treatment. Across 3 groups: p<0.0001*. RBC-Cy5 vs. IP-DEX: p=0.0002*
RBC-DEX vs. IP-DEX: p=0.6
RBC-DEX vs. RBC-Cy5: p=0.0007*
How Much is Enough? The association between physical activity and cardiometabolic health in adolescents

Karishma Sriram, Hillary Mulder, Heather Frank, Taruni Santanam, Ashley C Skinner, Eliana M Perrin, Sarah C Armstrong, Eric D Peterson, Michael Pencina, Charlene A Wong

Background: Physical activity has multiple health benefits for adolescents. However, by all measures, adolescents in the United States are not participating at the recommended levels of moderate or vigorous physical activity on a daily basis. Only 20% of a nationally representative sample of adolescents met current physical activity guidelines, which are at least 60 minutes of moderate or vigorous physical activity daily—about 420 minutes per week—yet little is known about the actual association between physical activity levels and measures of cardiometabolic among adolescents.

Methods: We examined adolescents (ages 12-19) from the 2003-2016 National Health and Nutrition Examination Surveys. Primary exposures were objective (accelerometer-based) and self-report (recreational) weekly mean minutes of moderate/vigorous PA. Primary cardiometabolic outcomes were systolic blood pressure (BP), diastolic BP, total cholesterol, high density lipoprotein (HDL), percent of body mass index (BMI) 50th percentile, and maximal oxygen uptake (VO2 max). The dose-response relationship between each exposure and outcome was assessed with unadjusted spline analyses and evaluated for non-linearity using natural cubic splines. Inflection points were determined for non-linear relationships and differences in cardiometabolic measures were estimated as function of amount of physical activity, adjusting for sociodemographic characteristics.

Results: Objective and self-report physical activity generally had linear relationships with cardiometabolic measures. For objective activity, non-linear relationships with inflection points at 90 minutes were identified for females for BMI and SBP. With adjustment, a 150-minute activity increase was significantly associated with a lower BMI (7.63%) and SBP (2.61mmHg). For males, non-linear relationships had inflection points at 150 minutes for BMI, SBP, and VO2 max. With adjustment, a 150-minute activity increase was significantly associated with a lower BMI (7.72%), HDL (2.04 mg/dL higher), and VO2 max (7.05 higher).

For self-reported activity, inflection points for DBP for females was found at 375 minutes. At 420 minutes of activity, the following significant differences in measures were identified: BMI (1.45% lower), DBP (1.59 mmHg lower), and HDL (1.01 mg/dL higher). For males, inflection points were identified for SBP at 500 minutes. At 420 minutes of activity, HDL was 1.16 mg/dL higher for males.

Conclusions: Among adolescents, physical activity was linearly associated with multiple cardiometabolic measures, supporting continued efforts to increase physical activity opportunities for adolescents. Differences by sex as well as the minutes of activity at the inflection points suggest reconsideration of more nuanced physical activity guidelines in adolescents.
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Anesthesiology, Surgery, and Environmental Physiology Study Program (ASEP)

Biomedical Engineering and Surgery Study Program (BES)
The status of global hearing healthcare: A unified view among 33 interviewed experts.

Andre M. Agassi, Gavin M. Yamey, Hanna E. Huffstetler, Debara L. Tucci

Background: Hearing loss affects 6-8% of the world’s population, and hearing impairment contributes to difficulties in communication and learning. A 2017 World Health Organization (WHO) report estimates that unaddressed global hearing loss costs $750-790 billion annually. The prevalence of hearing loss is disproportionately concentrated in low-income and middle-income countries. It is estimated that up to half of all hearing loss can be averted by some form of prevention. By interviewing key stakeholders, the study aims to understand the landscape of global hearing health. We hypothesized that interviews with a diverse group of stakeholders in hearing health would identify gaps in knowledge and strategies that may inform future work to successfully reduce the burden of hearing loss globally.

Methods: A total of 33 key stakeholders participated in a semi-structured Skype interview. Interview audio was transcribed into text files then coded using grounded theory. To ensure reliability, a random sample of 11 transcripts (1/3 of the total number of transcripts) underwent double-coding by a trained research associate.

Results: Interviewed stakeholders identified the following barriers and opportunities: Low prioritization of hearing health, the barrier of awareness, the (perceived) barrier of information, the barrier of fully efficacious solutions, the barrier of cost, and hope for the future.

Conclusion: Key stakeholders outline the landscape of global hearing health in this study, including current barriers and opportunities to overcome those barriers. This discussion highlights the need for additional effort to reduce the currently unbridled burden of hearing loss globally. A standard definition for what classifies as disabling hearing loss in tandem with improved funding and awareness can raise the prioritization of hearing health.
**Wound infusion with ropivacaine and ketorolac for analgesia after cesarean delivery: A randomized, double-blind, placebo-controlled trial**

Emily Z Barney, BS, Christina D Pedro, MD, Brock H Gamez, MD, Matthew E Fuller, MS, Jennifer E Dominguez MD, MHS, Ashraf S Habib, MBBCh, MSc, MHSc, FRCA

**Background:** Optimizing maternal analgesia after cesarean delivery is critical for improving short and long-term outcomes of the mother and neonate. Currently, the gold standard regimen involves a multimodal approach with neuraxial morphine, regular non-steroidal anti-inflammatory drugs (NSAIDs), and regular acetaminophen. There is little data regarding whether local anesthetic wound infusion provides additional benefit when used in addition to this gold standard. We therefore performed this prospective, randomized, double blind study and hypothesized that wound infusion with local anesthetic plus a NSAID would result in significantly improved analgesia after cesarean delivery compared with placebo.

**Methods:** This was a prospective randomized double-blind study. We enrolled women scheduled to undergo cesarean delivery under spinal or combined spinal epidural anesthesia with standardized anesthetic and postoperative analgesic regimen. Women were randomized to receive wound infusion for 48 hours with ropivacaine 0.2% plus ketorolac, or placebo (normal saline). Study drugs were administered through a catheter placed intraperatatively below the fascia and administered in PACU (10-mL bolus followed by infusion with an elastomeric ON-Q pump set to deliver 5 mL per hour for 48 hours). The primary outcome of the study was pain score with movement (sitting in bed from a supine position) at 24 hours after surgery. Secondary outcomes included pain scores at rest at 24 hours, pain scores at rest and with movement at 2 and 48 hours, opioid consumption, time to first rescue analgesic, opioid-related side effects, and patient satisfaction with postoperative analgesia.

**Results:** 71 patients completed the study per protocol: 38 subjects were randomized to the placebo group and 33 subjects to the ropivacaine plus ketorolac group. Patient demographics and intraoperative characteristics were comparable between the two groups. There was no significant difference between the groups in the primary outcome of pain score with movement at 24 hours (median [IQR] 5 [3-7] placebo group vs. 5 [4-6] ropivacaine plus ketorolac group, p= .9386). There were also no significant differences between the groups in pain scores at other time points, or in total opioid consumption between the placebo and ropivacaine plus ketorolac group (median [IQR] 27.5 [15-45] vs. 15 [5-45] mg oxycodone equivalents, p= .1051). The placebo group required rescue analgesics more quickly than the ropivacaine plus ketorolac group (median [IQR] 660 [9-1496] vs. 954 [244-1710] minutes), but the difference was not statistically significant (p= .2991). Thirteen patients did not require rescue analgesics (8 out of 33 in the ropivacaine plus ketorolac group (24%), compared to 5 out of 38 in the placebo group (13%), p=0.3698).

**Conclusions:** Under the conditions of the study, there was no benefit of wound infusion with ropivacaine and ketorolac in women who had received intrathecal morphine and a multimodal analgesic regimen. Median opioid consumption was reduced by half with wound infusion from 24-48h, but this difference was not statistically significant. Further larger studies powered for opioid consumption beyond 24 hours, and dose response studies to determine the optimum local anesthetic wound infusion rate are needed.
The INTUIT Study: Investigating Neuroinflammation Underlying Postoperative Neurocognitive Dysfunction, Delirium, and Brain Connectivity Changes in Older Adults

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Background: Postoperative cognitive dysfunction (POCD) and postoperative delirium (POD) occur in up to 40% of older adults and are each associated with increased mortality and decreased quality of life. However, the mechanism behind these disorders is not clearly understood. One potential cause of POCD and POD is postoperative neuroinflammation. Previous studies have found that pro-inflammatory cytokines increase in both serum and CSF following surgery. Further, we have recently demonstrated that a decrease in monocyte chemoattractant protein (MCP-1) receptor expression on monocytes at 24 hours after surgery is seen in patients who later develop POCD. Neuroinflammation can also affect the default mode network (DMN), an interconnected set of brain regions that is active during internally focused tasks. Disrupted resting state functional connectivity in DMN regions have been observed in patients with POCD. The objective of the INTUIT study is to characterize the relationship between postoperative neurocognitive function, CSF inflammatory biomarkers (including monocytes and downregulation of MCP-1 receptors), and DMN activity/connectivity (measured by fMRI).

Methods: The INTUIT study will enroll 200 patients over a 4-year period. Patients are eligible if they are >60 years old, undergoing elective non-cardiac and non-neurologic surgery with a case duration of at least 2 hours, and not currently taking immunomodulatory therapy or anticoagulants. Patients will undergo neurocognitive testing <1 month before surgery and 6 weeks after surgery. Blood and CSF samples will be obtained immediately before surgery and 24 hours, 6 weeks, and 1 year after surgery. Resting state fMRI (rs-fMRI) will be obtained in 100 patients <1 month before surgery and 6 weeks after surgery. Delirium screens will be performed daily on postoperative inpatients.

Results: As of May 1, 2019, 112 patients have been enrolled. Baseline neurocognitive testing has been performed on 110 patients, and both blood and CSF samples have been obtained from 96 patients. Baseline rs-fMRI has been obtained on 64 patients. 97 patients remained in the hospital at least one night and were assessed for delirium. 24-hour postop blood and CSF samples were obtained from 88 patients. To date, 91 patients have returned for 6-week follow up: 91 completed neurocognitive testing, 79 provided blood and CSF samples, and 50 completed rs-fMRI scanning.

Conclusions: The INTUIT study is assessing the relationship between postoperative neurocognitive function, CSF inflammatory biomarkers, and DMN activity/connectivity. We expect that the degree of pro-inflammatory signaling is associated with a higher incidence of POCD, and with DMN connectivity/activity changes. If true, this finding would be an important step toward identifying potential therapeutic targets for the prevention and treatment of POD and POCD.
Hypercapnic Ventilatory Response as a Predictor of Postoperative Opioid-Induced Respiratory Depression: Exploratory Study of a New Technique

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Funding provided by Duke University Department of Anesthesiology

Background: Incidences of postoperative opioid-induced respiratory depression (OIRD) ranges from 0.5-41%, and traditional risk factors do not reliably predict patient outcome. A potential risk factor that has received little attention and exploration is low ventilatory chemosensitivity. This pilot study establishes a methodology to explore chemosensitivity as a risk factor for postoperative OIRD, as well as OIRD distribution across sleep and wakefulness.

Methods: Following IRB approval and written informed consent, patients underwent a presurgical sleep study and ventilatory tests to determine hypercapnic ventilatory response during hyperoxia and mild hypoxia with (HCVR_{REMI}) and without (HCVR_{BL}) remifentanil (effect site concentration, Ce, 0.7 or 2.0 ng/ml). Postoperatively, minute volume (MV), respiratory rate (RR), sleep data and transcutaneous PCO2 (P_{TCO2}) were collected from post-anesthesia care unit (PACU) admission until the following morning. Pulse oximetry oxygen saturation (SpO2) and opioid consumption were collected from PACU admission to hospital discharge. End points were incidences of RR < 60% of baseline, MV < 60% of expected MV, SpO2 < 90% (room air) or 92% (supplemental O2), P_{TCO2} > 50 mmHg, and central or obstructive apnea/hypopnea. Statistical analyses were conducted with a significance of α = 0.05.

Results: Nine patients were studied and analyzed (2 F:7 M, age 65±7.5 years). Ce of 0.7 and 2.0 ng/ml both generally depressed HCVR, but the degree of depression was highly variable. Overall, high event frequencies were observed only with lower HCVR_{REMI} (HCVR_{REMI} < 1.00 and HCVR_{REMI} < 2.00 during hyperoxia and hypoxia, respectively). Linear regression showed negative correlations between OIRD frequency and HCVR_{REMI}, more strongly seen with Ce = ng/ml. OIRD events were significantly more likely to occur during sleep than wakefulness, particularly light sleep (p < 0.05 for SpO2 desaturation, central apnea and obstructive apnea). When adjusted for time spent in each wake or sleep stage, event frequency was still greater during sleep, with a tendency to occur during light and rapid eye movement (REM) sleep, but with significance only for obstructive apnea.

Conclusion: Ventilatory chemosensitivity may be an important predictor of OIRD. This study lends a quantitative and new perspective to classify risk for OIRD and details a methodology in which to pursue this investigation for future studies. Preliminary results suggest that high HCVR_{REMI} may be protective against OIRD, while lower HCVR determined by Ce = 2.0 ng/ml correlates with a greater frequency of OIRD events. OIRD events are more likely to occur during sleep, particularly REM and light sleep.
Impact of Sarcopenia and Body Mass Index on Clinical Response and Treatment-Related Toxicity of Pembrolizumab Treatment in Patients with Advanced Melanoma

Janice Hu BA MSc, Christel Rushing BS MSc, Sin-Ho Jung PhD, April Salama MD, Brent Hanks MD PhD, and Paul Mosca MD PhD MBA

**Background:** The recent emergence of immune checkpoint inhibitor therapies such as pembrolizumab, which targets the programmed death-1 receptor, has improved the survival outlook for patients with advanced melanoma. Recent retrospective studies have revealed a possible “obesity paradox” in which higher BMI is associated with better survival in melanoma patients treated with immunotherapy. Sarcopenia, defined as muscle failure due to adverse changes that accrue across a lifetime, has been shown to be a reliable predictor of prognosis in patients with stage III melanoma and has been associated with worse toxicity and lower survival rates in response to immunotherapy. The goal of this study was to investigate whether two patient body parameters—BMI and a simple measure for sarcopenia—correlate with toxicity and response to pembrolizumab in the treatment of advanced melanoma.

**Methods:** This was a retrospective study of 158 patients who underwent pembrolizumab treatment at Duke University Hospital from January 2014 to September 2018. Baseline characteristics, treatment, outcomes, and survival data were recorded. CT imaging at treatment start was evaluated to determine simple psoas muscle cross-sectional area and density. Patients in the lowest sex-specific tertile of psoas area were defined as sarcopenic, whereas patients in the lowest sex-specific tertile of density were defined as density-sarcopenic. Response to pembrolizumab was assessed using CT or PET (positron emission tomography) scans. A patient was recorded as experiencing a treatment-limiting adverse event (TLAE) if symptoms led to the permanent discontinuation of pembrolizumab. Univariate analysis was performed using logistic regression models. Overall survival and progression-free survival curves were analyzed using the Kaplan-Meier method.

**Results:** Sarcopenia and density-sarcopenia did not significantly correlate with TLAE, response, or survival. Patients who were obese had 4.9 times the odds of experiencing a TLAE (p<0.001). Having a low creatinine (less than 0.6 for females and 0.9 for males) was associated with 2.7 times the odds of experiencing a TLAE (p=0.044). Patients who had normal-high albumin levels had 3.3 times the odds of disease control compared to patients with low albumin (p=0.015). BMI and sarcopenia did not impact overall survival or progression-free survival.

**Conclusion:** These findings indicate that, while psoas cross-sectional area does not appear to predict clinically relevant outcomes, other readily available baseline characteristics such as albumin, creatinine, and BMI may be useful biomarkers to help guide the management of patients undergoing pembrolizumab treatment for advanced melanoma.
**Efficacy of Talimogene Laherparepvec (T-VEC) Therapy in Patients with In-Transit Melanoma Metastasis Decreases with Increasing Lesion Size**

Sabran J Masoud, Janice B Hu, Georgia M Beasley, John H Stewart IV, Paul J Mosca

*PolkaDot Mama Melanoma Research Foundation Travel Award*

**Background:** In-transit (IT) melanoma represents a distinct disease pattern occurring in 4-10% of melanoma patients, whereby disease recurs as dermal or subcutaneous nodules between the primary tumor site and regional lymph node basin. A significant proportion of these patients develop concurrent nodal or distant disease. Intralesional therapy has provided the means to treat to previously inaccessible IT lesions of the head, neck, and trunk, while portending fewer systemic side effects relative to regional chemotherapy. Talimogene laherparepvec (T-VEC) was the first oncolytic virus approved as first-line intralesional therapy for advanced locoregional melanoma, with a reported overall response rate (ORR) of 25% and complete response rate (CRR) of 10% in clinical trials. However, the role of patient selection in driving clinical response to therapy is not well defined. Here, we ascertain the role of patient selection on outcomes in a contemporary clinical practice setting, including the impact of clinical stage, other systemic therapy, and IT lesion diameter on clinical response.

**Methods:** Medical records were extracted for patients with recurrent Stage IIIB - IV in-transit melanoma completing T-VEC at Duke University Medical Center between January 1, 2016 and September 1, 2018. Patients were treated by one of three surgeons within a multidisciplinary group, each administering T-VEC in accordance with manufacturer guidelines. Kaplan-Meier analysis assessed time to clinical complete, partial, progressive, or no response, evaluated by serial measurements of lesion diameter. Logistic regression measured associations of clinicopathologic staging, maximum in-transit lesion diameter, T-VEC treatment regimen, and use of prior or concurrent therapy with ORR and CRR.

**Results:** Of 27 patients receiving T-VEC, 4 (14.8%) had stage IIIB disease, 16 (59.3%) stage IIIC, and 7 (25.9%) stage IV. Most patients (n = 24, 88.8%) also received non-operative systemic or locoregional therapy prior to, concurrently, and/or after receiving T-VEC. In this sample, 10 patients (37.0%) achieved complete response and 11 (40.7%) overall response at a median 22 weeks (95% CI 2.0 - 41.9 weeks and 15.8 - 28.2 weeks, respectively). Logistic regression demonstrated that increasing maximum lesion diameter predicted decreased ORR (odds ratio [OR] 0.866 (95% CI 0.753 - 0.995), p = .04), but did not predict decreased CRR (OR 0.900 (0.804 - 1.007), p = .067). Stage IV disease (OR 0.04 (0.00 - 0.74), p = .031) and history of PD-1 inhibitor treatment (OR 0.06 (0.01 - 0.74), p = .028) also predicted reduced clinical response.

**Conclusions:** Response rates to T-VEC, in an out-of-trial cohort, may be higher than previously suggested. While application of T-VEC therapy in overall lower stage patients, in the setting of other systemic therapy likely play significant roles, maximum in-transit lesion diameter is a rapidly assessed and useful indicator of successful T-VEC therapy. Prospective multi-center trials would be advisable prior to implementing lesion diameter among formal patient selection criteria.
Poster #7

Postoperative Analgesic Utilization after Cesarean Delivery in the United States: A Retrospective Cohort Study

Sydney E Reed, Matthew Fuller, Vijay Krishnamoorthy, Tetsu Ohnuma, Karthik Raghunathan, Ashraf S Habib

Background: Cesarean delivery (CD) is one of the most common operations performed in the US. Adequate pain control during and after this surgery is a top priority for patients and can reduce the risk of chronic pain and postpartum depression. Currently, the gold standard for analgesia following CD includes neuraxial morphine with scheduled acetaminophen and NSAIDs. Further, recent studies recommend scheduled acetaminophen with as-needed opioids in lieu of acetaminophen-opioid combination drugs to reduce opioid consumption, optimize analgesia, and reduce the risk of exceeding the recommended maximum daily dose of acetaminophen. However, it is unknown how common these practices were across the US. As such, the objective of our study was to describe postoperative analgesic practice after CD in the US.

Methods: A retrospective cohort study was performed using an inpatient administrative database from 2008-2014 (Premier Inc., Charlotte, NC). Women aged 18 years who had undergone CD (ICD-9 code 74.x) under spinal or epidural anesthesia (by charge codes for anesthesia type) were identified. Patients with charge codes for general anesthesia or narcotic medications with unclear route of administration were excluded. In addition, patients who died during hospitalization were excluded. Descriptive statistics were used to examine demographic and clinical characteristics, facility characteristics, classes of analgesics administered, and utilization patterns over time.

Results: 393,327 women were included in the analysis. Analgesics administered during admission are tabulated below. Overall, only 4.0% of patients received neuraxial morphine plus NSAIDs and acetaminophen. However, this proportion increased over the duration of the study from 1.4% in 2008 to 8.3% in 2014. The most common practice was to administer neuraxial morphine, NSAIDs, and acetaminophen-opioid combination drugs (62.2%).

Conclusion: Most patients received NSAIDs and neuraxial morphine for postoperative analgesia, although no more than 10% received what is currently considered the gold standard. Acetaminophen-opioid combination medications were administered much more commonly than acetaminophen alone, representing an area for improvement in postoperative analgesic practice following CD. Further investigations should be performed to analyze more recent trends in postoperative analgesic practice following CD and to examine outcomes related to implementation of recent recommendations.

<table>
<thead>
<tr>
<th>Analgesics</th>
<th>Analgesic Combinations</th>
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<tr>
<td>Neuraxial Morphine</td>
<td>NSAIDs + Acetaminophen</td>
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<td>NSAIDs</td>
<td>NSAIDs + Acetaminophen- Opioid</td>
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<tr>
<td>Acetaminophen</td>
<td>NSAIDs + Acetaminophen- Opioid</td>
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<tr>
<td>Acetaminophen- Opioid</td>
<td>Oral Opioids</td>
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<td>Oral Opioids</td>
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Data are presented as number (%).
Thrombosis in Infants in the Neonatal Intensive Care Unit: Analysis of a Large National Database

Victoria Robinson, Meredith Achey, Kristy Pahl MD, Rachel Greenberg MD, Elisabeth Tracy MD

Background: We conducted the largest retrospective study to date investigating the incidence and management of thromboembolism in the Neonatal Intensive Care Unit (NICU) population. Recent studies have suggested that the incidence of thromboembolism in neonates is rising, and that it should be considered in the differential for children with a suspicious clinical picture. It has also been shown that the incidence of thrombosis is higher in neonates specifically, than in older children. We know that in neonates, the high morbidity and mortality associated with thromboembolism means that early recognition and treatment is critical in this vulnerable population. Infants in the NICU setting are already at higher risk because they are often medically fragile or critically ill. We hypothesize that there is a subgroup of infants in the NICU that is at increased risk of arterial and venous thromboembolism. Identifying and studying this group will help us better recognize and ultimately treat these infants.

Methods: In this retrospective study, records for all infants admitted to a Pediatrix NICU from 1997 through 2015 were queried for venous and arterial thromboembolism. We assessed demographic characteristics and known risk factors including sepsis, pressor requirement, mechanical ventilation, and invasive procedures. Characteristics for infants who developed thromboembolism were compared with those who did not using the Pearson X2 test for categorical variables and Wilcoxon rank-sum test for continuous variables. Stepwise logistic regression was used to identify factors associated with neonatal thrombosis.

Results: Primary outcome was incidence of venous or arterial thromboembolism. We assessed the correlation of clinical characteristics and demographics with incidence of thromboembolism, and evaluated characteristics of treatment. Of 1,158,755 infants, 2367 (0.20%) were diagnosed with thromboembolism. In a multivariable regression analysis, early gestational age, low birth weight, male sex, congenital heart disease, sepsis, ventilator requirement, pressor requirement, central venous catheter, and invasive surgery were associated with thromboembolism. While a majority of infants (74%) with diagnosed thromboembolism received no anticoagulation, we identified higher prevalence of anticoagulant use in infants with thromboembolism than those without (26% vs. 0.2%, p<0.001). Type of anticoagulant therapy was variable. Overall mortality and length of stay were increased in infants with thromboembolism compared to others.

Conclusions: The need to better understand, predict, and treat neonatal thromboembolism grows more urgent as our ability to intervene in critically ill infants improves. With advancing technology, more of the smallest and most critically ill infants will be able to survive, and will likely require more surgery and invasive procedures. This in turn will likely continue to increase the incidence of venous and arterial thrombosis in infants, making diagnosis and management of the infants more critical. Although our study is limited in its scope and ability to distinguish between types and locations of thromboses, it is the largest study to date and points to the need for further, more granular studies. Age-related differences in neonatal hemostasis and thrombosis make it even more important to evaluate the safety and efficacy of currently used therapies, and develop novel therapeutics and treatment strategies specific to this population.
Improved Compliance with Early Sepsis Management is Associated with Shorter Hospital Stay and Safer Care

Larissa G. Rodriguez-Homs, MBS, Sabran J. Masoud, BS, Matthew J. Mosca, Oliver K. Jawitz, MD, Harry R. Phillips, MD, Cara O’Brien, MD, Paul J. Mosca, MD/PhD/MBA

Background: Medicare requires public reporting of hospital compliance with the Sepsis and Septic Shock Early Management (SEP-1) bundle. To inform improvement work, we aimed to determine whether hospital-to-hospital variation in SEP-1 compliance is linked to hospital size and measures of safety and operational efficiency. The objective was to determine whether SEP-1 bundle compliance is associated with hospital size and indices of operational excellence, including patient safety index (PSI-90), average length of stay (ALOS) and readmission rate.

Methods: Data was obtained from Medicare’s Hospital Compare online database and Centers for Medicare & Medicaid Services Files. Records missing SEP-1 data were excluded. Pearson correlation, controlling for staffed beds, and an independent t-test were used for analysis.

Results: In 2,653 acute care hospitals, SEP-1 score was inversely associated with staffed bed number (r = -.144, p < .001), PSI-90 (r = -.097, p < .001), and ALOS (r = -.134, p < .001). There was no association with 30-day readmission rate (r = -.02, p = .31). Hospitals in the lowest versus highest quartile by bed number had SEP-1 compliance score of 49.79 20.17% vs. 46.91 16.82%, p < .001. Hospitals in the lowest versus highest quartile for SEP-1 score had an average length of stay of 5.02 1.16 days vs 4.69 1.09 days and PSI-90 rate of 1.03 0.22 vs. 0.98 0.16, p < .001 for both.

Conclusions: In this study of Medicare publicly reported data, SEP-1 score was inversely associated with hospital size, PSI-90 score and average length of stay. While this study does not establish a causal relationship, it supports the hypothesis that ability of hospitals to successfully implement SEP-1 is associated with superior performance in key measures of operational excellence.
Liver Function and Inflammation During Normothermic Machine Perfusion: Can We Expand the Donor Pool Even Further?

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R. Randall Bollinger Scholarship, American Society of Transplant Surgeons Presidential Student Mentor Grant

Background. The main obstacle facing the field of liver transplantation is a critical shortage of donor organs. Normothermic machine perfusion (NMP), which perfuses the graft with oxygenated perfusate at physiologic temperature, is a promising emerging technology to allow use of more high-risk organs by reducing ischemic injury sustained during graft transport from donor to recipient and by enabling assessment of graft function before implantation. However, damage associated molecular patterns (DAMPs) released during NMP are known to activate inflammatory signaling and contribute to poor outcomes. Our project aimed to measure levels of DAMPs and their inflammatory effects during NMP and relate them to donor traits, as well as to characterize potential biomarkers of graft viability during NMP.

Methods. We measured DAMPs in perfusate collected during NMP of 14 human livers, including HMGB1 by ELISA, other proteinaceous DAMPs using LC/MS/MS untargeted proteomics, and extracellular DNA (exDNA) using PicoGreen reagent. We measured toll-like receptor (TLR) activation caused by DAMPs in perfusate using reporter cell assays. We measured levels of clinical biochemical markers using standard point-of-care clinical tests. We also measured levels of ischemia-reperfusion-injury-associated microRNAs in perfusate using qPCR. These data were correlated with donor characteristics and recipient outcomes.

Results. Levels of exDNA increased over time during perfusion, although activation of TLR9, primarily sensitive to cell-free DNA, did not. Levels of HMGB1 decreased over time, with corresponding decrease in activation of the proteinaceous-DAMP-sensing TLR4. Levels of HMGB1 were lower for older donors ($R^2=0.65$, $p=0.0005$). However, levels of exDNA were higher in livers donated after cardiac death (DCD grafts), and levels of the DAMPs heparan sulfate and S100 proteins increased more during perfusion in DCD livers. However, DAMP levels and TLR activation did not correlate strongly with recipient outcomes. The best predictors of 0 to 7 day post-operative peak AST and ALT levels were change in AST and ALT levels, respectively, from the start to the end of perfusion (AST: $R^2=0.64$, $p=0.0006$; ALT: $R^2=0.82$, $p<0.0001$); however, DCD livers had low peak post-op transaminase levels despite having some of the highest levels in perfusate. Per fusate ALT levels at the end of perfusion predicted ICU stay ($R^2=0.49$, $p=0.006$) and post-op hospital length of stay ($R^2=0.75$, $p<0.0001$). Levels of the hepatocyte-derived miR122 and miR148a and cholangiocyte-derived miR222, miR30e, and miR296 in perfusate were higher for DCD livers and for the livers from the donors with the highest BMIs, but levels decreased over time during perfusion and did not predict post-op outcomes as well as did perfusate AST and ALT levels.

Conclusions. Our results show that multiple DAMPs are released during NMP, especially by organs donated after cardiac death, although in our limited sample size DAMP release did not correlate with outcomes. Transaminase and microRNA levels in perfusate predict peak post-op serum AST—an accepted marker for long-term graft and patient survival—and ALT levels, and transaminases predict length of stay as well. These data provide valuable insight into inflammatory activity in the NMP system and demonstrate the utility of NMP as a platform to assess graft function and predict post-transplant outcomes based on clinical testing of perfusate.
Surgery for Femoroacetabular Impingement Syndrome Improves Strength, Flexibility, and Postural Stability

Nnamdi Amilo, Peter Howard, Mallory Faherty, Arakua Welbeck, Daniel Le, Carolyn Killelea, Caroline Westwood, Richard C. Mather III, Timothy Sell

Background: Femoroacetabular impingement syndrome (FAIS), a potentially debilitating condition of the hip, is characterized by abnormal articulation between the femoral head and the acetabulum. Despite a recent increase in the number of people receiving surgical correction, there is a lack of high-level evidence demonstrating its efficacy as well as its functional effects on the hip. Therefore, characterizing how surgical correction of FAIS affects the hip joint would be beneficial. The aim of this study was to examine and compare hip strength, flexibility, and static postural stability in a cohort of subjects undergoing hip surgery for FAIS. It was hypothesized that surgical repair would result in significant improvement in all measures.

Methods: Twelve subjects (2 males, 10 females, age: 35.1 ± 9.3 years, height: 166.8 ± 8.0 cm, weight: 71.5 ± 13 kg) with unilateral FAIS were tested prior to surgery and at six months. Hip strength in flexion, extension, adduction, abduction, internal rotation, and external rotation was measured using a handheld dynamometer. Hip flexibility was assessed by measuring flexion, extension, adduction, abduction, internal rotation and external rotation active range of motion (aROM) using an inclinometer. Single-leg postural stability was assessed with both eyes-open and eyes-closed conditions on a force plate. All tests were performed bilaterally. Comparisons were made prior to surgery and at six months following surgery for the involved leg only. Depending on normality, either a paired t-test or a Wilcoxon rank-sum test was used. Statistical significance was set a priori at p<0.05.

Results: Six months following surgery there was significant improvement in internal rotation aROM (p=0.010), flexion aROM (p=0.025), flexion strength (p=0.008), abduction strength (p<0.001), adduction strength (p=0.001), internal rotation strength (p<0.001), extension strength (p=0.003), external rotation strength (p=0.006), and the static postural stability eyes-closed composite score (p=0.023). Static postural stability with eyes open as well as aROM of hip extension, external rotation, adduction and abduction were not significant between time points.

Conclusions: Surgical repair of FAIS resulted in improvements in hip strength, flexibility and static postural stability at the six-month time point. The results of this study may help clinician-patient discussions regarding expectations and provide insight into appropriate rehabilitation strategies.
The Role of Malnutrition in 90-day Outcomes After Total Joint Arthroplasty

Collin S. Black, Daniel E. Goltz, Sean P. Ryan, Amanda N. Fletcher, Samuel S. Wellman, Michael P. Bolognesi, Thorsten M. Seyler

Background: Research has linked malnutrition to more complications in total joint arthroplasty (TJA) patients. The role of preoperative albumin measurement in predicting length of stay (LOS) and 90-day outcomes remains understudied. Often, an albumin cutoff ≤3.5 g/dL is used as proxy for malnutrition, although this value remains understudied. This pre-operative level may be missing some patients at risk for adverse events post TJA. As arthroplasty moves toward a bundled payment environment, predicting post-operative complications is increasingly important. However, the best preoperative albumin cutoff level for predicting poor post-operative outcomes remains understudied.

Methods: Total joint arthroplasty patients at a single institution from 2013 to 2018 were reviewed for preoperative albumin level. 4,047 cases (TKA: 2058, THA: 1989) had available data. Review included 90-day readmissions rate, 90-day ED visit rate, postoperative length of stay (LOS), and rate of discharge to SNF. Additionally, the Youden function was employed to determine the optimal cutoff point on the Receiver Operator Characteristic (ROC) curve for predicting 90-day readmission rate in a univariable model.

Results: 5.6% of patients experienced a readmission within 90 days and 9.6% of patients had at least one ED visit within 90 days. Overall prevalence of malnutrition was 3.6%, and this cohort experienced a longer average length of stay (3.5 vs 2.2 days; p < 0.0001) and was more likely to experience a readmission (16% vs 5%; p < 0.0001) or ED visit (18% vs 9%; p = 0.0005). Additionally, an albumin of ≤3.5 g/dL was correlated with more frequent discharge to SNF/rehab (30.8% vs 14.7%; p < 0.0001), increased risk for 90-day readmission with univariable and multivariable logistic regression-(OR: 1.79; p < 0.0001) and (OR: 1.55; p < 0.0001) respectively and 90-day ED visits-(OR: 1.62; p < 0.0001) and (OR: 1.35; p < 0.0001) respectively. The optimal albumin cutoff was found to be 3.94 g/dL in a univariable model for 90-day readmission.

Conclusions: Screening for malnutrition may serve an increasingly important role in preoperative evaluation for total joint arthroplasty. We found low albumin level as a proxy for malnutrition is associated with a significant increase in postoperative LOS, 90-day adverse outcomes, and more frequent discharge to SNF. Finally, an albumin cutoff value of 3.5 g/dL may be missing some at-risk patients but further research is necessary to find the optimal value.
Personalized Beat Anomaly Detection in Wearable ECGs through Deep LSTM Transfer Learning

Joshua D’Arcy, Ric Telford
Barr-Spach Medicine and Engineering Scholarship

Background: Wearable health technology is a rapidly growing field. With sensors capable of continuously monitoring electrogram signals, heart rate, and other biometric data, a vast dataset of unlabeled health data is generated daily. The wearable ECG is one of the fastest growing wearable consumer technologies available today, and there exist growing needs to detect anomalies in unlabeled ECG data. This work aims to generate an adaptive algorithm capable of 1) rapidly modeling a personalized ECG signal with the limited computational power found on wearable devices and 2) reliably detecting arrhythmic behavior using that model.

Methods: By training a deep long short-term memory (LSTM) recurrent neural network model on 36 hours of normal sinus ECG signals from the MIT-BIH Normal Sinus Rhythm database, a general model for detecting normal sinus activity was produced. Then, using computationally lightweight transfer learning techniques to rapidly retrain the model on patient-specific signals from the MIT-BIH Arrythmia database, a personalized normal sinus model was produced. This personalized model was then set to the task of identifying arrhythmic behavior (PVCs and APBs) from normal sinus behavior for each patient. A third, naïve model was also trained on specific patient data to further demonstrate the power and rapid training of the transfer learning model. The methods used for preprocessing the data were intentionally designed to require only the limited amount of computational power found on wearable devices, and included only simple baseline wander correction, standardization, and resampling. An anomaly detection algorithm generated error vectors from the three models, and an optimal threshold for arrythmia detection was set for each using differential evolution. F-scores, precision, recall, and true positive / false positive rates were used to determine performance.

Results: The personalized, rapidly-retrained model achieved F2 scores of .863 and .918 for detecting PVC’s and APB’s, respectively. It outperformed the general model with an F2 increase in detecting PVC’s (weighted F2 score +.065, n = 20, p = .002) and APB’s (weighted F2 score +0.256, n=8, p = .018). The TPR/FPR ratio was over 13 times higher in the personalized model than the general model in detecting APB’s. The optimum threshold (log10p(i)) for arrhythmia detection had a lower weighted average (-7.4 versus - 4.7 for PVC’s and -21.5 versus -7.7 for APB’s). The personalized model also outperformed the naïve model for detecting PVC’s (weighted F2 score +.081, n = 20, p = .001) and APB’s (weighted F2 score +252, n = 8, p = .012). The TPR/FPR was 12 times higher than the naïve model for detecting APB’s. The optimum threshold for the transfer learning model had a lower weighted average than the naïve model (-7.4 versus -4.6 for PVC detection and -21.5 versus - 7.2 for APB detection). Importantly, although the training set was identical for the naïve and transfer learning models, the naïve model took substantially longer to train and yielded inferior results.

Conclusions: This sensitive and lightweight model for real-time ECG anomaly detection, when paired with a specific and more computationally heavy model for classification and diagnosis, could be an effective means for outpatient ECG analysis. Overall, it appears that transfer learning has a role in rapidly personalized arrhythmia detection in wearable ECG technology.
BET and HDAC inhibition: a potent pairing against soft tissue sarcoma

Charlton Tsai, David Kirsch

Background: Management of soft tissue sarcoma (STS) remains challenging due to high rates of recurrence and poor sensitivity to traditional chemotherapy agents. In recent years, epigenetic-modifying agents such as bromodomain and extraterminal motif protein (BET) inhibitors and histone deacetylase (HDAC) inhibitors have shown promise in a variety of leukemias and solid tumors, with early evidence suggesting they may synergistically interact via action on the anti-proliferative Hippo signaling pathway. The purpose of this study was to determine whether BET/HDAC inhibition might also be efficacious against STS.

Methods: To evaluate the efficacy of BET/HDAC inhibition in STS, the BET inhibitor JQ1 and non-selective HDAC inhibitor SAHA (vorinostat) were tested on 2 murine STS cell lines and in a primary mouse model of STS. To investigate whether a subset of HDAC proteins (of which there are 11 total) is responsible for the anti-proliferative action of HDAC inhibitors, a CRISPR/Cas9 genetic knockout screen of individual HDAC genes was conducted in vitro, along with a pharmacologic screen of selective HDAC inhibitors in vitro and in vivo. To explore whether the Hippo pathway might play a role in BET/HDAC inhibitor action, mRNA and protein levels of critical pathway components were quantified in treated cells and tumors.

Results: JQ1 and SAHA synergistically inhibited cellular proliferation in vitro and slowed tumor growth in vivo. A genetic knockout screen failed to identify any individual HDAC as indispensable for tumor cell proliferation; however, a pharmacologic screen identified Class I (HDAC1, 2, 3, 8) inhibitor romidepsin as an efficacious agent in vitro, with nearly 1000-fold greater potency than SAHA. While romidepsin showed decreased efficacy as a single agent in vivo compared to SAHA, it did demonstrate potential synergy with JQ1, delaying tumor growth and prolonging survival when used in combination. Mechanistically, JQ1/SAHA and JQ1/romidepsin combination treatment decreased mRNA and protein levels of the pro-growth transcriptional activators YAP1 and TAZ, both in vitro and in vivo; these proteins are classically inhibited by the Hippo pathway.

Conclusions: Combination BET/HDAC inhibition may be an effective and synergistic therapeutic strategy for STS patients. Selective Class I HDAC inhibition may be sufficient for synergy with BET inhibition. Synergy between BET and HDAC inhibitors may be mediated through reactivation of Hippo pathway signaling.
An Examination of the Relationship between Fear of Reinjury and Neuromuscular, Musculoskeletal, and Biomechanical Characteristics at the Time of Return to Sport following Anterior Cruciate Ligament Reconstruction

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Background: Kinesiophobia (KSP) is linked to reinjury risk and diminished function after return-to-sport (RTS) following anterior cruciate ligament reconstruction (ACLR). The purpose of this study was to compare individuals with high KSP to low KSP across modifiable neuromuscular, musculoskeletal and biomechanical characteristics at time of RTS. Identifying differences between ACLR groups based on KSP may help with development of strategies to reduce KSP and reinjury risk.

Methods: 19 subjects (11 males, 8 females; mean age=21.0±7.1 years; mean Tegner Activity Level pre-injury=7.9±1.2), all cleared for RTS post-unilateral ACLR, participated in this study (10.8±2.0 months post-ACLR). Individuals were grouped by KSP scores (<17; n=7 and ≥ 17; n=12) using the Tampa Scale for Kinesiophobia-11. Comparisons were made between groups in lower extremity flexibility and strength, isokinetic knee strength, static balance, dynamic postural stability, and stop-jump on the ACLR limb. Limb symmetry index comparisons were made in isokinetic knee strength and hop tests.

Results: Average KSP score for the low KSP group was 15.6±0.8, while that of the high KSP group was 20.6±3.2 (p<0.001). All measures otherwise did not demonstrate statistically significant differences between the two groups (p>0.05).

Conclusion: No differences in neuromuscular, musculoskeletal and biomechanical function were identified between low and high KSP subjects. Further data collection is necessary to confirm findings; however, these results provide data to power future investigation.
Post-Norwood Pulmonary Artery Shear Stress and Coronary Perfusion from Patient-Specific 3D CFD Simulations Based on Routine Clinical Data

Michael Kaplan, PhD, Piers Barker, MD, Kevin Hill, MD, Reid Chamberlain, MD, James Chen, PhD, Amanda Randles, PhD
Duke University Department of Medicine Stead Scholarship Program

Background: Hypoplastic left heart syndrome (HLHS) is a congenital heart defect that is lethal in the neonatal period without staged surgical palliation. Although significant surgical improvement has occurred over the years, the first surgical stage, known as the Norwood Procedure, continues to result in significant morbidity and mortality. Interstage mortality can be caused by problems with post-operative coronary perfusion. A major source of long-term morbidity for these patients is due to inadequate pulmonary artery growth and the formation of pulmonary artery stenoses. Pulmonary artery pathology has been shown to be correlated with wall shear stress, a quantity that is most precisely measured through computational fluid dynamic (CFD) simulations. Consequently, optimization of the Norwood Procedure can be studied by generating CFD models to investigate pulmonary artery wall shear stress and coronary perfusion. However, computational fluid dynamic models of HLHS often rely on imaging modalities that are not part of the standard treatment course for most patients. Generating an adequately large sample size to retrospectively study the correlation between hemodynamics and clinical outcomes after the Norwood Procedure necessitates relying exclusively upon routine clinical data. We present a methodology based only on routine clinical data which enables us to generate the first patient-specific 3D CFD models of flow in the pulmonary arteries, aorta, and coronary arteries after the Norwood Procedure. We then use this methodology to investigate how different choices for surgical shunt configuration change pulmonary artery wall shear stress and coronary perfusion.

Methods: Two Duke post-Norwood patients were retrospectively selected. Patient 1 has a modified Blalock-Taussig (mBT) shunt and Patient 2 has a Right Ventricle to Pulmonary Artery (RVPA) shunt. Biplane cineangiography is used to reconstruct patient anatomy. Patient-specific pressure tracings, Qp/Qs, and Doppler echocardiography is used to determine the model boundary conditions. Time-varying 3D flow is calculated using HARVEY, a massively parallel lattice Boltzmann approach to the Navier-Stokes equations. Varying shunt types, sizes, and the inclusion of a valve is performed by “virtual surgery” to explore how these factors alter coronary perfusion and wall shear stresses, specifically the time averaged wall shear stress (TAWSS) and oscillatory shear index (OSI).

Results: Consistently lower values of OSI occur for patients who received an mBT shunt compared to an RVPA shunt. Unlike OSI, TAWSS is found to be more sensitive to patient characteristics than shunt characteristics. The models also demonstrate that shunt size has a smaller effect on TAWSS and OSI than shunt type. Additionally, the models show that the effect of a valve in the RVPA is to decrease both OSI and TAWSS. Coronary perfusion is found to be sensitive to patient-specific factors, such as the pulmonary artery resistance, in addition to the surgical shunt characteristics.

Conclusion: A novel method for generating patient-specific 3D CFD models of congenital heart disease is presented. OSI is found to be predominantly determined by shunt type, while TAWSS and coronary perfusion are more sensitive to patient-specific factors. With an improved understanding of coronary perfusion and pulmonary artery shear stress, this methodology can be applied to optimize the Norwood Procedure for a given patient.
Clinical Research Training Program (CRTP)
Clinical Research Study Program (CRSP)
Decreasing Time Spent in the Waiting “Rheum”: How eConsults Improve Access to Rheumatology

Zachary J. Brandon; John Paul Shoup, MD; Robert T. Keenan, MD MPH MBA; Alex H. Cho, MD MBA; Teresa K. Tarrant, MD; Elizabeth Malcolm, MD; Kevin P. Shah, MD MBA

Background: While the importance of timely rheumatologic care is known, long lead times and poor completion rates of rheumatology referrals often prevent appropriate care. Of patients referred to rheumatology from Duke Primary Care (DPC), only 59% are scheduled and only 40% arrive to their appointments, with a prolonged median wait of 77 days. Prior studies have demonstrated eConsults ability to improve efficiency and coordination of specialty care, along with the potential to decrease wait times. To increase rheumatology referral completion rates and decrease wait times we implemented E-Communications (or eConsults) to rheumatology. PCP and specialist satisfaction of eConsult implementation and use were also tracked.

Methods: Two Duke Primary Care (DPC) practices initially participated in the eConsult program, with expansion to 2 additional practices after 6 and 8 months respectively. In lieu of traditional referrals, primary care providers place an eConsult request. Each eConsult is reviewed within 72hrs by a rheumatologist who provides recommendations for additional workup or treatment. The rheumatologist recommends either continued management by the primary care provider or triaged in-person rheumatologic evaluation. The eConsult completion rates and turnaround time were monitored. Conversion rates of eConsult requests to in-person appointments and wait times for these appointments were tracked. The eConsults were compared with traditional referrals from DPC clinics during the intervention period. Surveys of both primary care and rheumatology participants following the pilot program assessed participant satisfaction.

Results: Over 12 months, 49 primary care providers submitted 230 eConsults for 212 individual patients. 58.4% (n=124) of patients were recommended for in-person evaluation. Of these, 6% (n=8) were recommended for evaluation within 7 days, 26% (n=32) within 30 days, and 68.5% (n=85) within 90 days or no priority. 86.5% (n=199) of eConsults were completed within the target 72hrs (median response 15.5hrs, IQR 4.1hrs-33.5hrs). Compared to DPC patients referred to rheumatology during the same time period, eConsult patients had shorter wait times (median 31.2d vs. 77d). The eConsult patients were significantly more likely to be scheduled (89.5% vs. 59.3%, X² = 45.1, p < 0.001) and complete their in person specialty evaluation (78.2% vs. 40.2%, X² = 68.2, p<0.001). We found high levels of PCP satisfaction with eConsults. When compared to standard referrals, all PCP respondents rated eConsults as “5- much better” (79%, n=11) or “4- somewhat better” (21%, n=3). PCP perception of overall care coordination improved significantly post-implementation (Likert scale 1-2 negative, 3-5 neutral/positive, X² = 19.7, p<0.001). Rheumatologists had positive experiences as well, with an overall satisfaction mean of 4.2 out of 5 on a Likert scale (IQR 4-5).

Conclusions: Through implementation of eConsults, we improved access and increased visit completion within a high demand specialty. The eConsult service was positively received by both PCPs and specialists and perceived care coordination improved.
Lifetime Psychosocial Stress Exposure Associated with Hypertensive Disorders of Pregnancy

Madeleine Caplan, Lauren Keenan-Devlin, PhD, Alexa Freedman, PhD, William Grobman, MD, Pathik Wadwha MD, PhD, Claudia Buss PhD, Gregory Miller, PhD, Ann Borders, MD

Background: Hypertensive disorders of pregnancy complicate 5-10% of all pregnancies and are a major cause of pregnancy-related morbidity. Exposure to psychosocial stress has been associated with systemic inflammation and adverse birth outcomes in pregnant women. Thus, it is probable that psychosocial stress and inflammation play a role in the development of hypertensive disorders of pregnancy. The primary objective of this analysis was to determine if a woman’s lifetime psychosocial stress exposure was associated with an increased risk of hypertensive disorders of pregnancy. Additionally, we examined whether serum inflammation was an underlying biological mediator for this relationship.

Methods: A multi-site prospective study was conducted in a socio-demographically diverse cohort of 647 pregnant women. In a study visit between 12 and 20 6/7 weeks’ gestation, maternal psychosocial stress was assessed with six validated assessments and inflammation was measured via log-transformed serum concentrations of INFγ, IL-10, IL-13, IL-6, IL-8, and TNFα. A composite stress score was calculated for each participant from the six stress assessments. The diagnosis of a hypertensive disorder of pregnancy was abstracted from the medical record, and was defined as the presence of gestational hypertension after 20 weeks of pregnancy and/or preeclampsia. The association between composite stress and hypertensive disorders of pregnancy was determined using binary logistic regression. Inflammation, using the six inflammatory biomarkers, was tested as a potential mediator between stress and hypertensive disorders of pregnancy.

Results: Participants with higher composite stress scores were more likely to develop hypertensive disorders of pregnancy (OR 1.50, 95% CI 1.06 – 2.12). When adjusted for known risk modifiers of hypertensive disorders, including maternal age, race/ethnicity, parity, pre-pregnancy BMI, diabetes, chronic hypertension, and smoking during pregnancy, the risk remained relatively unchanged (OR 1.50 95% CI 1.03 – 2.20). No mediation effect by inflammation was observed.

Conclusions: Independent of known risk factors, women exposed to greater composite stress burden across the life course are at increased risk of developing hypertensive disorders of pregnancy.
The Impact of Malnutrition on Outcomes in Patients Undergoing Transjugular Intrahepatic Portosystemic Shunt Insertion

Ryan S. Chiang, Alice Parish MSPH, Donna Niedzwiecki PhD, Matthew R. Kappus MD, Andrew J. Muir MD MHS

**Background:** Malnutrition is a common comorbidity in patients who suffer from chronic liver disease. Prior research has shown malnutrition to be associated with poor post-operative outcomes after hepatic resection and liver transplantation. Transjugular intrahepatic portosystemic shunt (TIPS) is a procedure performed for refractory complications of cirrhosis. The purpose of this study is to assess the impact of malnutrition on the outcomes for patients undergoing TIPS.

**Methods:** A retrospective analysis was performed using the Healthcare Cost and Utilization Project: National Inpatient Sample database to assess a representative sample of admissions in the United States who had a TIPS procedure from 2005-2014. Primary outcomes included in-hospital mortality and length of stay (LOS) post-procedure. Predictors of mortality were assessed by survey weighted logistic regression. Age, gender, race-ethnicity, admission type (elective vs non-elective), insurance payer, hospital region, malnutrition, comorbidities, total LOS, and race-ethnicity by malnutrition interaction were included as predictors in the model. Survey weighted Chi-squared and t-tests were used for comparison of categorical and continuous variables, respectively.

**Results:** From 2005-2014, an estimated 53,207 (95% CI 49,330-57,085) total admissions with TIPS procedures were performed. 11% of admissions had an accompanying diagnosis of malnutrition. 15% of patients with malnutrition died post-procedure, compared to 11% of patients without malnutrition (p-value < 0.001). 22% of malnourished patients required transfer to a skilled nursing facility upon discharge compared to 10% of patients without malnutrition. Patients with malnutrition had a longer post-procedural LOS with a median of 6.7 days (Q1, Q3: 2.8, 13.6), compared to 2.9 days (Q1, Q3: 1.0, 6.7) among patients without malnutrition (p-value <0.001). Patients without malnutrition had a median total hospital charge of $79,781 (Q1, Q3: $47897, $143074) while patients with malnutrition had significantly higher costs with a median of $144,752 (Q1, Q3: $82226, $263555) (p-value<0.001). Patients with a diagnosis of malnutrition had 1.31 (95% CI 1.07, 1.59) times the odds of mortality compared to patients without malnutrition after controlling for other factors. Additionally, patients of Black race-ethnicity had 1.65 (95% CI 1.29-2.10) times the odds of mortality compared to NH White patients. Non-elective admission patients had 2.27 (95% CI 1.88-2.75) times the odds of mortality compared to elective admission patients after adjusting for other factors.

**Conclusions:** Malnutrition had a significant impact on patients who underwent TIPS. They suffered from longer length of stay, higher total hospital charges, and increased in-hospital mortality after TIPS insertion. Malnutrition, non-elective hospital admission, and Black race-ethnicity were significant predictors of mortality when controlling for other factors. Further research is needed to evaluate the consequences of poor nutrition status associated with TIPS, and the capability of nutritional optimization to improve outcomes.
Model Ensembling vs Data Pooling: Alternative ways to analyze data across Duke University Health System

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*Duke Institute for Health Innovation Clinical Research and Innovation Scholarship*

**Background:** Postoperative complications arise in 15% of all US surgical procedures, with high-risk surgeries having complications in up to 50% of cases. Understanding which Duke University Health System (DUHS) patients are at high-risk is a critical unmet need. DUHS consists of three hospitals: Duke University Hospital (DUH), 957 beds, Duke Regional Hospital (DRH), 369 beds, and Duke Raleigh Hospital (DRAH), 186 beds. Using our machine learning post-operative risk prediction models, we determined how models best performed within the three hospital settings by using different combinations of training hospital population cohorts.

**Methods:** We compared LASSO and extreme gradient boosted decision tree models (XGBoost) models. We tested on the same site versus models trained on all sites versus ensembled models trained from each of the three sites where the predictions from each site-specific model are averaged for a test patient.

**Results:** Table 1 depicts DUHS patient population characteristics. Table 2 shows the accuracy results of experiments across DUHS patients.

**Conclusion** Combining data from multiple Duke Health hospitals can increase model performance for both LASSO and XGBoost. However, ensemble methods demonstrate equal performance. Ensembling allows for models to explicitly learn from each individual hospital, whereas data pooling does not. These experiments and results have large implications for our health care delivery system, distributed research networks, as well as addressing issues around health data privatization. If ensemble modeling is a feasible way to leverage information across network sites, pooling patient health information may not be required for machine learning model development. Individual sites may choose to build local models that health systems and distributed research networks can then ensemble and scale across networks.

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<th>Training Cohort</th>
<th>Testing Cohort</th>
<th>XGBoost AUROC</th>
<th>XGBoost AUPROC</th>
<th>LASSO AUROC</th>
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*Table 1: DUHS patient population characteristics.*

*Table 2: Accuracy results of experiments across DUHS patients.*
**Cause-Specific Mortality in a Population-Based Cohort of 7,289 Individuals Diagnosed with Lymphoplasmacytic Lymphoma/Waldenström Macroglobulinemia, 2000-2016**

Nicole H. Dalal, Graça M. Dores, Rochelle E. Curtis, Martha S. Linet, Lindsay M. Morton

*NIH Medical Research Scholars Program Fellowship*

**Background:** Population-based disease registries represent a rich resource for cancer research, allowing for the study of rare diseases and outcomes. Information on cancer incidence and mortality is otherwise difficult to ascertain from clinical trial data due to a limited number of enrollees and short follow-up time. Lymphoplasmacytic lymphoma (LPL) and Waldenström macroglobulinemia (WM) are indolent lymphomas for which effective treatment is available, but for which data on cause-specific mortality are lacking. Understanding cause-specific disease mortality for LPL/WM patients can help identify high-risk patient subgroups, latency patterns, and potentially treatable or preventable comorbidities that can be targeted to impact survival favorably and inform priority areas for the allocation of healthcare resources.

**Methods:** We identified 7,289 adults diagnosed with incident first primary LPL (n=3,108) or WM (n=4,181) during 2000-2016 among 17 US population-based cancer registries. Patients were followed from the time of LPL/WM diagnosis until death, loss to follow-up, or end of study (December 31, 2016), whichever occurred first. We identified 3,132 deceased patients and their causes of death based on death certificates. We estimated cumulative mortality, accounting for competing risks of death for up to 16 years following LPL/WM diagnosis; excess absolute risks (EARs) of death per 10,000 person-years; and standardized mortality ratios (SMRs), calculated as the ratio of observed versus expected number of deaths in a given category, to determine the relative risk of death compared with the general population. We tested for statistically significant (P<0.05) heterogeneity of SMRs between patient subgroups using Poisson regression models stratified by sex, lymphoma subtype (LPL versus WM), receipt of initial chemotherapy, age at diagnosis, and time since diagnosis.

**Results:** The 16-year cumulative mortality for lymphomas, non-lymphoma cancers, and non-cancer causes was 23.2%, 8.4%, and 14.7%, respectively, for patients aged <65 at diagnosis of LPL/WM; 25.9%, 11.6%, and 34.2%, respectively, for those aged 65-74 years; and 33.4, 11.2%, and 48.7%, respectively, for those aged ≥75 years. Compared with the general population, overall, LPL/WM patients had a 20% higher risk of death due to non-cancer causes (n=1,341 deaths, SMR=1.2; 95% confidence interval [CI], 1.1-1.2), most notably from infectious (n=188; SMR=1.8; 95%CI, 1.6-2.1), respiratory (n=143; SMR=1.2; 95%CI, 1.0-1.4), and digestive (n=80; SMR=1.8; 95%CI, 1.4-2.2) diseases, but no excess mortality from cardiovascular diseases (n=477, SMR=1.1; 95%CI=1.0-1.1). Risks were highest for non-cancer causes within one year of diagnosis for all patients with LPL/WM (n=239; SMR<1year=1.3; 95%CI, 1.2-1.5), and declined thereafter (n=522; SMR≥5years=1.1; 95%CI, 1.1-1.2). The overall SMR for myelodysplastic syndrome/acute myeloid leukemia was notably increased (n=46; SMR=4.4; 95%CI 3.2-5.9), whereas solid neoplasm deaths were not elevated, except among ≥5-year survivors (n=145; SMR=1.3; 95% CI=1.1-1.5). SMRs were generally higher among younger individuals and were similar between LPL and WM patients.

**Conclusions:** We provide the first estimates of cause-specific mortality risks for LPL/WM compared to the general population, creating a framework for optimizing follow-up care strategies, including an increased focus on non-cancer mortality, especially within the first year of LPL/WM diagnosis. With mortality due to non-cancer causes and non-lymphoma cancers surpassing mortality due to lymphomas among older individuals, early recognition and interventions may favorably impact mortality risks, especially for older patients.
How Much is Enough? The Dose Relationship of Physical Activity on Cardiometabolic Health for Young Adults

Heather Frank, Hillary Mulder, Karishma Sriram, Taruni Santanam, Ashley C Skinner, Eliana M Perrin, Sarah C Armstrong, Eric D Peterson, Charlene A Wong

Background: The 2018 Physical Activity (PA) Guidelines recommend 150 minutes weekly for adults. It is unclear if this activity level matches cardiovascular benefits for young adults (YA) transitioning from adolescence. We aim to determine the dose-response of physical activity on measures on cardiometabolic health measures in young adults.

Methods: We examined YA (20-29 years) in the 2003-2016 National Health and Nutrition Examination Survey. Primary exposures were objective (accelerometer) and self-report weekly mean minutes of moderate/vigorous physical activity (PA). Primary cardiometabolic outcomes were body mass index (BMI), high-density lipoprotein (HDL), total cholesterol, systolic blood pressure (SBP), diastolic blood pressure (DBP), and maximal oxygen uptake (VO2 Max). The dose-response relationship between each PA exposure and cardiometabolic outcome was assessed with unadjusted spline analyses; each pairing was tested for non-linearity using natural cubic splines, with non-linear relationships converted to 2-part piece-wise linear splines. Sex stratified outcomes were modeled using multivariable linear regression adjusting for sociodemographic characteristics. Mean estimated differences are presented for 150-minute PA dose increases.

Results: Among females, most dose-response relationships were linear. Compared to no minutes, 150 minutes of objective activity was associated with a lower BMI (1.42 kg/m^2, p=0.011) and total cholesterol (5.43 mg/dL, p=0.033), while 150-minutes of self-reported activity was associated with an HDL of 1 mg/dL higher (p=0.003) and DBP 0.42 mmHg lower (p=0.023). Among males, inflection points in the dose-response curves occurred for objective activity with BMI and VO2 Max at approximately 100 minutes. Compared to no minutes of activity, 150 objective activity minutes was associated with a higher VO2 max (7.89 ml/kg/min, p<0.001), while 150 minutes of self-reported activity was associated with a lower BMI (-0.26 kg/m^2, p=0.002), higher HDL (0.52 mg/dL, p=0.027), and lower total cholesterol (-1.35 mg/dL, p=0.016).

Conclusions: Among young adults, numerous linear and curvilinear associations between physical activity and cardiometabolic health measures exist. BMI and HDL yielded the most consistently significant relationships in young adults, with substantial differences by sex and activity measurement methods. Our study extends to younger adults the evidence on the association of physical activity and cardiometabolic health among adults. The many linear relationships in young adults’ support activity guideline recommendations for increases in physical activity, while variability by sex and activity measurement type informs future activity guidelines.
Barriers and Facilitators to Performing CPR in Hemodialysis Clinics: A Qualitative Study

Samuel A. Hofacker, Laura J. Fish, Margaret B. Falkovic, and Patrick H. Pun

**Background:** Sudden cardiac arrest is the leading cause of death among hemodialysis patients, and occurs frequently in outpatient dialysis clinics. A previous study showed that dialysis staff-initiated CPR was associated with a significant increase in patient survival when compared to EMS/First Responder-initiated CPR, yet rates of staff-initiated CPR are suboptimal, with CPR not being initiated by dialysis staff in 19% of cases. The aim of this study was to identify barriers and facilitators to performing CPR in outpatient hemodialysis clinics.

**Methods:** We recruited a convenience sample of staff from dialysis clinics across the state of North Carolina to participate in a survey about their experience and confidence with CPR in dialysis clinics. 10 survey respondents completed in-depth interviews about their experience, knowledge, training, and practices regarding CPR in dialysis clinics. Participants included 5 nurses, 1 nurse manager, and 4 patient technicians, and represented 2 large, for-profit organizations and 2 not-for-profit organizations. Thematic analysis was used to identify salient themes regarding factors influencing delivery and quality of CPR in dialysis clinics.

**Results:** Through our in-depth interviews, we identified three broad themes: 1) contextual factors to performing high quality CPR in dialysis clinics; 2) procedural challenges in applying the cardiac arrest chain of survival to the dialysis clinic, 3) the need for continuous improvement programs for CPR in dialysis clinics. Regarding contextual factors that impact the quality of CPR in dialysis clinics, participants described the chaotic environment of the dialysis clinic during a cardiac arrest, challenges with the physical layout of the clinic, the importance of knowing a patient's DNAR status, and the long term relationships formed between dialysis patients and providers. With respect to procedural challenges, participants discussed the difficulty of recognizing cardiac arrest in patients who are frequently asleep or hypotensive, uncertainty regarding how to deal with the dialysis machine during a cardiac arrest, and concern about how to best position a patient for CPR (i.e. in the dialysis chair, with or without a backboard, or on the floor). Participants described a lack of consistent protocol for performing CPR in dialysis clinics and emphasized the importance of having structures in place to help improve the quality of CPR in dialysis clinics. Such structures included hands-on training, particularly mock codes, established team roles and leadership during CPR, and a debriefing process following a cardiac arrest event.

**Conclusion:** The dialysis clinic setting presents unique challenges to performing high quality CPR following cardiac arrest. Our findings suggest considerable room for improvement regarding resuscitation policies, procedures, and training. We propose a dialysis clinic-specific cardiac arrest chain of survival, that in addition to the elements of the AHA out-of-hospital cardiac arrest chain of survival, includes the additional elements of recognizing cardiac arrest, dealing with the dialysis machine, and positioning the patient. Our study calls for further investigation into how to optimize and standardize the protocol for performing CPR in outpatient hemodialysis clinics, and offers insight into potential avenues for continuous improvement of CPR in dialysis clinics.
Acoustic Analysis of Left Ventricular Assist Devices

Cameron R. Olsen, BS, Ravi Karra, MD, Boyla O. Mainsah, PhD, Leslie M. Collins, PhD, Priyesh A. Patel, MD

Background: Left ventricular assist devices (LVAD) are a life-saving therapy for patients with advanced heart failure, yet clinical evaluation and management of these patients remains challenging. Standard surveillance tools, such as the stethoscope, have not been designed nor optimized for managing these patients, as the mechanical nature of the LVAD pump creates substantial noise that obscures the native sounds of the heart, limiting the ability to ascertain valve function and hemodynamics during a bedside physical exam. In addition, it is unknown to what extent the LVAD pump affects the native sounds of the heart. We propose that by obtaining digital stethoscope recordings of the heart-LVAD system, and by applying advanced filtering and signal processing techniques, we can separate native heart sounds from LVAD noise and obtain important information regarding the function of the heart and LVAD pump.

Methods: We performed an observational, single-center, prospective study at Duke University Hospital to assess whether we could distinguish LVAD sounds from underlying normal heart sounds. Twelve (12) hospitalized patients on LVAD therapy with either a HeartWare LVAD or HeartMate 3 LVAD were enrolled and underwent a routine LVAD speed optimization study with echocardiography, during which heart sounds were recorded using two Thinklabs One digital stethoscopes placed at both upper sternal borders. Each stethoscope signal was independently analyzed and then correlated with information obtained via echocardiography.

Results: We successfully distinguished underlying native heart sounds from overlapping LVAD noise. Adaptive filtering resulted in cleaner signals with significantly diminished LVAD sounds. Both S1 and S2 were successfully identified via correlation with echocardiographic information. Features of these heart sounds are within the typical ranges seen in healthy patients, and features such as S2 peak amplitude and peak frequency correlated weakly with changes in the pump speed ($r = -0.16, 0.21$ and $p = 0.01, 0.001$). Changes in the frequency of aortic valve closure were also identified and correlated to valve closure seen on echocardiography.

Conclusions: Acoustic analysis of patients undergoing LVAD therapy resulted in the identification of unique pump and native heart sound features. Such features may prove useful in developing future machine learning algorithms for pump optimization and complication detection.
Depression is Associated with Degree of Cognitive Decline After Surgery in Older Adults with Postoperative Cognitive Dysfunction

Deborah Oyeyemi, BA, Heather E. Whitson, MD MHS, Jeffrey N. Browndyke, PhD, Harvey Jay Cohen, MD, Joseph P. Mathew, MD, Miles Berger, MD PhD

Pfizer Foundation Grant and the Duke Clinical & Translational Science Institute Fellowship, Karen L. Wrenn Alzheimer’s Travel Award, National Medical Fellowships Alzheimer’s or Dementia Related Care Scholarship

Background: Up to 40% of older adults undergoing surgery are at risk of developing postoperative cognitive dysfunction (POCD), generally defined as a > 1 or 2 standard deviation (SD) drop in cognitive performance from before to >1 month after surgery. Older adults also frequently develop postoperative delirium, an acute form of cognitive impairment that is associated with preexisting depression. Whether depression is associated with POCD is less clear. Thus, here we evaluated the association between POCD and depression/anxiety symptoms in older adults.

Methods: 110 non-cardiac, non-neurological surgical patients at Duke University Medical Center aged 60 years or older underwent cognitive testing, Center for Epidemiologic Studies-Depression (CES-D), and State-Trait Anxiety Inventory-State (STAI) instrument screening before and 6 weeks after surgery. Cognitive scores were combined by factor analysis into four cognitive domains; the means of these domain scores yielded the continuous cognitive index (CCI), a representation of overall cognitive function. POCD as a dichotomous outcome was defined as 1-SD drop in ≥1 cognitive domain from before to 6 weeks after surgery. Postoperative cognitive change severity was defined as CCI change from before to after surgery, with a negative CCI change indicating POCD and a positive CCI change indicating improved postoperative cognitive function.

Results: Of 103 participants with complete data, 27.2% (n=28) met criteria for POCD. Baseline measures of anxiety and depression symptoms were not associated with cognitive change from before to after surgery across the whole patient cohort. In older adults who experienced POCD, greater preoperative depression was associated with lesser POCD severity (Spearman’s r = 0.60, p<.001). Worsened depression from before to after surgery was associated with increased POCD severity (Pearson’s r = -0.57, p=0.002). Over 1 in 4 patients who developed POCD screened positive for depression before surgery.

Conclusion: Our findings suggest that in patients who developed POCD, higher baseline depression severity was associated with less postoperative cognitive impairment, while increases in depressive symptoms after surgery were associated with more severe postoperative cognitive impairment. The mechanisms underlying these results are unclear, though it is possible that unmeasured latent variables (perhaps such as blood brain barrier dysfunction) may predispose some patients to both worsened postoperative depression and more severe POCD. Future POCD research in older adults should incorporate depression screening measures, adjust for baseline depression severity, and investigate whether depressive symptoms may be a modifiable risk factor in POCD.
Poster #26

ACCEPT (Assessing Colonization vs. Clearance after Probiotic instillation) – Bio-K Plus Probiotic Validation Study

Emily L. Romanoff B.S., Zhiqing Huang M.D., Liping Fend, M.D./Ph.D., and Nazema, Siddiqui, M.D., M.H.Sc.

Funded by Duke Claude D. Pepper Older Americans Independence Center

Introduction: There is an urgent need to investigate non-antibiotic methods of preventing and treating recurrent urinary tract infection (rUTI), particularly in postmenopausal women. In microbiome studies, lactobacilli are associated with urinary tract health, but are decreased in older women. Decreased urinary lactobacilli has been suggested as a risk factor for rUTI. Therefore, methods of augmenting lactobacilli are being explored as treatment options for rUTI for older women. Oral and vaginal probiotics have not successfully decreased UTI frequency. However, probiotic instillations delivered into the bladder have not yet been explored. In order to develop an instillation therapy, pre-clinical testing is needed. Our primary objective was to quantify and characterize the growth of lactobacillus from Bio-K Plus probiotic capsules in vitro. Furthermore, we aimed to confirm the absence of bacterial contaminants to ensure patient safety in future clinical testing.

Methods: Bio-K Plus contents were dissolved under sterile conditions in 50 mL of saline, followed by incubation at room temperature until plating. Solutions containing the contents of one capsule versus two capsules were tested in parallel. A total of 6 incubation time intervals were tested, varying from 30 minutes to 24 hours according to published logarithmic growth patterns of reconstituted Lactobacillus spp. After the desired time had elapsed, 100 μL of probiotic suspension were cultured on Lactobacillus MRS agar plates under aerobic conditions at 37°C for 60 hours. All plates were imaged and colony-forming units (CFUs) were quantified for each incubation time period. Colonies were randomly chosen and further cultured in liquid MRS broth for later species testing. To test for contaminants, DNA was extracted from four probiotic suspensions using the Qiagen DNeasy Blood and Tissue kit with lysozyme. DNA was quantified using Qubit and submitted for 16S rRNA gene sequencing. Recovered bacterial sequences were identified at the genus level, placed into taxonomic tables for further qualitative review, and data were reviewed to assess for proportions of genera other than Lactobacillus.

Results: When reconstituted in room temperature saline under aerobic conditions, Lactobacilli from Bio-K Plus probiotic capsules were recovered at a mean of 17.9 billion CFU (one capsule) and 23.4 billion CFU total (2 capsules), with some variation depending on incubation time. Two-way ANOVA showed that there were no statistically significant differences in CFU recovery between one versus two capsules (p=0.25). When comparing incubation times, CFU recovery was significantly higher at 4 hours compared to other timepoints (p=0.04). After 4 hours of incubation, CFU recovery declined in both one and two capsule solutions. Recovered colonies from dissolved Bio-K+ had a uniform appearance on plates, suggesting one bacterial genus, which was further confirmed using 16S ribosomal RNA gene sequencing. The proportion of bacteria belonging to the Lactobacillus genus ranged from 99.93% to 99.94% across the four sequenced samples.

Conclusions: Based on these data, an ideal instillation agent would contain 1 dissolved capsule, left in room temperature saline for 4 hours. Results from 16S rRNA gene sequencing show minimal bacterial contaminants. Therefore, reconstituted Bio-K+ probiotic appears safe for future human trials to deliver a highly concentrated solution of live Lactobacillus directly into the bladder via instillation therapy.
**“Real-World Experience with Electronic Patient Reported Outcomes and Missing Items: “Don’t Ask Me Irrelevant Questions”**

Heather A. Rosett; Susan C. Locke, PhD; Steven P. Wolf, MS; Kris W. Herring, PhD, MScM; Gregory P. Samsa, PhD; Jesse D. Troy, PhD; Thomas W. LeBlanc, MD, MA, MHS

**Introduction:** Patient-reported Use of electronic patient-reported outcomes (ePROs) in cancer care can improve quality of life and prolong survival by enhancing the detection and tracking of unmet supportive care needs. However, there remain unanswered questions about how to handle missing data in real-world implementation. We hypothesized that patients may skip specific questions they feel do not apply to them. To assess this, we examined the relationship between patient demographics and missing items in a real-world ePRO dataset.

**Methods:** We utilized a prospectively collected database of ePROs from oncology clinics administering Patient Care Monitor 2.0 (PCM), a validated symptom survey of 78 items for men and 86 for women. We tabulated the frequency of missing items by item and domain (emotional, functional and symptom-related), and examined these by age, gender, education, race and marital status.

**Results:** In 20,986 encounters there were responses to at least 1 PCM item from 6933 patients. On average just 1% of items were skipped per encounter. By domain, 12.3% of functional, 8.4% of symptom-related, and 1.6% of emotional constructs contained at least one missing item. The highest frequency of item non-response was seen in older patients (>60yo) and those with high school education or less. The most frequently skipped items included: “attend a paid job” (10.7%), “reduced sexual enjoyment” (3.8%), and “running” (3.7%) (Table 1). These questions may be less relevant to older or retired individuals, who quit running years earlier, or who have never been avid runners. Men had less missingness overall, except for “cooks for self” and “house work”, which may reflect traditional gender roles in the Southeast US.

**Table 1: Most Frequently Missing Items**

<table>
<thead>
<tr>
<th>Item</th>
<th># Missing Items (% of Total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Attend paid job</td>
<td>2237 (10.7%)</td>
</tr>
<tr>
<td>Reduced sexual interest</td>
<td>793 (3.8%)</td>
</tr>
<tr>
<td>Run</td>
<td>781 (3.7%)</td>
</tr>
<tr>
<td>Heavy work or activity</td>
<td>542 (2.6%)</td>
</tr>
<tr>
<td>House work</td>
<td>517 (2.5%)</td>
</tr>
<tr>
<td>Driving</td>
<td>487 (2.3%)</td>
</tr>
<tr>
<td>Cook for self</td>
<td>484 (2.3%)</td>
</tr>
<tr>
<td>Run errands</td>
<td>470 (2.2%)</td>
</tr>
<tr>
<td>Weight gain</td>
<td>414 (2.0%)</td>
</tr>
<tr>
<td>New lump / mass</td>
<td>390 (1.9%)</td>
</tr>
</tbody>
</table>

**Conclusions:** In a real-world ePRO implementation, items pertaining to more universal issues for cancer patients, like emotional well-being, have much lower rates of missingness, especially compared to functional items like “attend a paid job.” These results suggest that patients differentially complete ePROs based on perceived question relevance to them. The underlying driver behind individual item non-response may itself be an important data point in clinical care, warranting further study and discussion during clinic visits.
Demographic Representation in 17-P Preterm Birth Prevention Studies: A Systematic Review

Megan Smith, BA; Geeta Swamy, MD; Sarahn Wheeler, MD

Background: There are multiple studies evaluating 17α-hydroxyprogesterone caproate (17-P) for recurrent preterm birth (PTB) prevention. Persistent racial, ethnic, and geographic disparities exist in US PTB rates and the NIH publishes reporting guidelines race and ethnicity in clinical research. The demographic reporting patterns across studies evaluating 17-P is unknown. Our study examines race, ethnic, and geographic representation in 17-P PTB prevention studies.

Methods: Following PRISMA systematic review guidelines, we identified studies evaluating 17-P for PTB prevention published between January 2000 and July 2018. Original research examining IM 17-P for PTB prevention in singleton gestations beginning at 16-20 weeks and reporting PTB outcomes were included. We excluded non-English publications. Publications meeting inclusion criteria were manually abstracted for full-text analysis. Two independent reviewers evaluated articles for inclusion/exclusion criteria adherence. Descriptive statistics were used to describe patterns in racial, ethnic, and geographic reporting.

Results: We identified 624 articles based on the initial literature search and ultimately included 26 studies meeting the inclusion/exclusion criteria. Of the included studies, 14 (53.8%) were conducted within the US and 12 (46.2%) were international. The majority of US studies (n=13, 92.9%) reported participants’ race and half (n=7) reported ethnicity. A total of 8,623 participants were enrolled in US 17-P studies in which at least one racial/ethnic category was reported including 1,108 (11.8%) white, 2,460 (28.5%) black or African American, 26 (3.0%) Asian participants, and 661 (7.7%) participants of Hispanic ethnicity. Compared to US recurrent PTBs, there was a significantly lower proportion of white, Asian, AI/AN, and NH/PI participants enrolled in studies and a significantly higher proportion of black/African American participants. The proportion of Hispanic participants was significantly less than the population experiencing recurrent PTB in the US (7.7% vs. 21.5%, p < 0.0001). Nine studies included “other” as a race category, totaling 217 (2.5%) participants. None of the studies included a mixed race or multiracial category. In studies reporting at least one race category, there was a total of 6,443 (74.7%) participants that were not classified under any race category and otherwise left out of race reporting. Precise geographic location is unclear for the majority of participants (n=6,808, 77.6%) as US multicenter studies did not report specific location of participants.

Conclusions: Current 17-P literature lacks comprehensive reporting of participant demographics and fails to adhere to reporting minimums defined by the NIH. Participants are frequently grouped into an “other” race category that serves to classify those not identifying within a single race, or otherwise are left unspecified and unreported. Only half the US studies reported ethnicity and Hispanic enrollment was low relative to the population experiencing PTB. We are unable to report if 17-P for PTB prevention has been studied in the geographic regions with the highest PTB rates. This collective lack of valuable information hinders the ability to detect variation in 17-P efficacy by race, ethnicity or geography.
Catheter ablation of atrial fibrillation in patients with diabetes mellitus

Allen Wang, BS; Tracy Truong, MB; Cindy Green, PhD; Eric Black-Maier, MD; Jonathan Piccini, MD, MHS

Background: Diabetes mellitus (DM) is an independent risk factor for atrial fibrillation (AF). DM patients with AF carry worse prognosis than those in sinus rhythm, and often have worse symptoms, lower quality of life, increased hospitalizations rates, and higher overall mortality. Catheter ablation is an established treatment for patients with symptomatic drug-refractory AF. However, few studies have evaluated the effect of diabetes on clinical outcomes after AF ablation. The primary purpose of this study was to compare ablation outcomes in patients with DM with the general population.

Methods: We performed a retrospective analysis of 351 consecutive patients who underwent first-time AF ablation. Clinical outcomes included freedom from recurrent atrial arrhythmia, symptom burden (Mayo AF Symptom Inventory [MAFSI]), and cardiovascular and all-cause hospitalizations at 12 months.

Results: Patients with DM (n = 65) were older, had higher BMI, more hypertension, had a higher CHA2DS2-VASC score, more persistent AF, and larger left atrial diameter. Median (Q1, Q3) total radiofrequency duration (64.0 minutes [43.6, 81.4] vs. 54.3 minutes [39.2, 76.4], p = 0.132) did not differ between DM and no-DM patients. After a median follow-up of 9.8 months, freedom from recurrent atrial arrhythmia was not significantly different between DM vs no-DM patients after adjustment for baseline differences (adjusted HR 1.39 [0.81, 2.38], p = 0.235), with similar improvements in the total MAFSI score (-6.5 vs -7.1, p = 0.575) at latest follow-up. The rates of cardiovascular (7.7% vs 10.5%, p = 0.193) and all-cause hospitalization (16.9% vs 14.1%, p = 0.559) at 12-month follow-up were similar between the two groups. There was a non-significant trend towards increased AF recurrence with higher HbA1c levels (HR 1.28 [0.93, 1.75], p = 0.131).

Conclusion: Catheter ablation of AF has similar efficacy in patients with diabetes compared to the general population, after adjusting for baseline differences. There were no significant differences in procedural characteristics, arrhythmia-free recurrence, symptom burden improvement, or hospitalization rates between patients with and without diabetes. Further studies are needed to determine the effect of glycemic control on ablation outcomes in this population.
Diving in the clouds: experimental validation of the Cross corrections to decompression tables for dives made at high altitude

Weis TW, Dong TW, Natoli MJ, Lance RM, Howle LE, Pieper CF, Freiberger JJ, Derrick BJ, Moon RE

Background: Decompression sickness (DCS), commonly known as “the bends,” is a serious and potentially fatal consequence of bubble formation within the body. Bubbles arise due to a rapid reduction in ambient pressure, such as the ascent from a SCUBA dive. In dives made at altitude, the reduced ambient pressure increases the risk of DCS. A method was designed by E.R. Cross in which the ratio of ambient pressure at sea level to ambient pressure at altitude is used to calculate a “virtual depth.” This virtual depth is greater than the actual depth, increasing the decompression requirement and creating a safety factor to account for the increased risk of DCS at altitude. However, this method has not yet been systematically tested.

Methods: This study applied the Cross corrections to US Navy decompression tables for 36 no-stop dives made from 8,000, 10,000, and 12,000 feet above sea level while breathing air or an oxygen-enriched gas.

<table>
<thead>
<tr>
<th>n =</th>
<th>Altitude (ft)</th>
<th>Acclimatization time (hrs)</th>
<th>Dive depth (fsw)</th>
<th>Time at depth (min)</th>
<th>Breathing gas</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>8,000</td>
<td>12</td>
<td>60</td>
<td>30</td>
<td>21% O2</td>
</tr>
<tr>
<td>16</td>
<td>10,000</td>
<td>48</td>
<td>60</td>
<td>30</td>
<td>21% O2</td>
</tr>
<tr>
<td>8</td>
<td>12,000</td>
<td>48</td>
<td>100</td>
<td>21</td>
<td>35% O2</td>
</tr>
</tbody>
</table>

Thirty six healthy volunteers age 18-40 were recruited. Subjects were screened with an ECG, PFTs, hemoglobin, pregnancy test, maximal oxygen consumption (exercise) test), pressure test, and physical exam. Prior to diving, all subjects acclimatized for 12 or 48 hours at simulated altitude in a hypo/hyperbaric chamber. Following acclimatization, subjects were fully submerged in a pool while breathing through a regulator and compressed to the respective depth. After diving, subjects returned to altitude where they were monitored for signs and symptoms of DCS. Additionally, subjects were scanned with 2-D precordial ultrasound to detect venous gas emboli (VGE).

Results: No divers displayed any signs or symptoms of DCS. One diver had Grade 3 VGE (at least one bubble per cardiac cycle). Binomial distribution analysis using the Jeffreys interval yields a 95% confidence interval with an upper limit of a 6.7% probability of DCS.

Conclusion: Although 32 more dives are needed to complete the study as designed, preliminary data suggest that the Cross corrections are a safe approach to diving at high altitude.
Continuous Glucose Monitoring (CGM) and Ambulatory Glucose Profile (AGP) Analysis during the First Trimester of Pregnancy

Qi Yu, Ling-Jun Li, MBBS, MMED, PHD, Kok Hian Tan, MBBS, MMed, FRCOG, FAMS
Duke-Singapore Student Scholar Fellowship

Background: Continuous glucose monitoring (CGM) is a minimally invasive device that has been increasingly adopted for use in the last two decades due to its comparable accuracy with self-monitoring of blood glucose (SMBG). More recently, flash glucose monitoring (FGM) systems have been developed without the inconvenience of calibration. These systems have not yet been used to collect ambulatory glucose profile (AGP) data from women in their first trimester of pregnancy. In this pilot study, we aimed to capture and analyze AGP data using both conventional and novel indexes during the first trimester of pregnancy.

Methods: We conducted an observational pilot study on pregnant women recruited from KK Women’s and Children’s Hospital in Singapore. We randomly allocated patients to Freestyle LibrePro with blinded reader or Freestyle Libre with non-blinded reader (1:1) for use up to 14 days. We computed the overall AGP mean glycemic level and mean amplitude of glycemic excursions (MAGE) for glucose variability, together with time in range (TIR) based on the area under the glycemic curve (AUC). According to local dietary practice, we defined hours for fasting (10pm-6am), lunch (11am-2pm), dinner (5pm-8pm) and random (6am-11am, 2pm-5pm, 8pm-10pm), respectively, and presented the time-specific glycemic variables using these intervals. We further developed a few novel indexes (i.e. TIR under arbitrary glycemic gradual cut-offs) in glycemic profiling.

Results: We collected AGP data from 24 patients (13 Libre, 11 LibrePro). We compared two device groups and found significant differences in overall mean glycemic level (Libre 4.24 vs LibrePro 4.66 mmol/L, p<0.0001). There were also strong correlations between mean MAGE and mean glycemic level (Pearson r = 0.8413, p <0.0001), and TIR>7.8mmol/L (Spearman 0.6927, p=0.0002).

Conclusions: Comparison of patients using Libre vs LibrePro suggests that patients with readers and access to their own glucose values had a significantly lower AGP mean glycaemia. Additionally, positive correlations between MAGE, 24hr mean glycemic level, and TIR>7.8mmol/L suggest that abnormally high levels of glycemic profiling is a combination of all patterns. We hope to further study these parameters of AGP data during the second trimester of pregnancy along with diagnosis of gestational diabetes (GDM).
The eye of the beholder: discrepancy between self-reported vision and visual acuity in patients with age-related macular degeneration.

Priscila P Cunha, Jonathan P Wright, David Madden, Guy G. Potter, Eleonora M Lad, Scott W Cousins, Heather E Whitson.

Supported by the National Center for Advancing Translational Sciences of the National Institutes of Health under Award Number TL1TR001116 and TL1TR002555

Background: Although visual acuity (VA) is used during clinical care of patients with age-related macular degeneration (AMD), AMD patients often report vision problems out of proportion with their VA. Our objective was to estimate the prevalence of discrepancy between VA and self-reported vision (SRV) and to evaluate factors that may contribute to the discrepancy.

Methods: We analyzed data from an observational study. Distance VA was measured with Snellen chart and converted to logMAR scale. SRV was assessed with a single question rating eyesight with both eyes on a scale of 0 to 5. VA/SRV discrepancy was defined consistent with previous literature. Contrast sensitivity (CS) was assessed by Pelli-Robinson chart. Type of AMD and information on presence of cataracts was abstracted from medical charts. Cognition was assessed using verbal fluency (FAS) test. Correlation analyses between VA and SRV and separately between CS and SRV were done using Spearman rank correlation, and Fisher r-to-z transformation was used to compare the correlation coefficients. Group differences were assessed with Chi-square tests (for proportions) and t tests (for continuous variables). Multivariate logistic regression adjusted for age and education was used to determine if verbal fluency was a predictor of discrepancy.

Results: Of 80 participants with AMD, 22 (27%) displayed VA/SRV discrepancy and of those, 20 (91%) reported SRV worse than VA. Discrepant and non-discrepant participants did not significantly differ based on gender, race, years of education, AMD type or presence of cataracts. Discrepant individuals were significantly younger than non-discrepant. The correlation coefficient between VA and SRV was -0.58, and between SRV and CS was 0.53. There was no significant difference between the absolute value of the two correlation coefficients (z=-0.36, p=0.36). Verbal fluency was not a predictor of discrepancy.

Conclusions: The discrepancy was not explained by any of the factors investigated. Both VA and CS were moderately correlated to SRV, but CS was not a superior indicator of SRV. Younger age was associated with discrepancy. Future research is needed to investigate other potential contributors to VA/SRV discrepancy not examined here, such as near visual acuity, timing and course of visual changes, and patient expectations and adaptation.
Quantifying Benefit-Risk Tradeoffs For Surgical Options In Low-Risk Thyroid Cancer

Maya Talbott MHS, Sara Ahmadi MD, Juan Marcos Gonzalez Sepulveda PhD, Shelby D Reed PhD, Jui-Chen Yang MEM, Randall Scheri MD, Michael Stang MD, Sanziana Roman MD, Julie Ann Sosa MD, MA

*Completed with support from the Duke CTSA TL1 Pre-Doctoral Scholarship.*

**Background:** Since 1975, the incidence of thyroid cancer has tripled. Surgery is the mainstay of treatment, and tradeoffs between the two surgical options (lobectomy and total thyroidectomy) are preference sensitive, yet patients’ perspectives have not been well studied. We designed a discrete-choice experiment (DCE) to quantify tradeoffs relevant to surgical options for low-risk thyroid cancer.

**Methods:** Participants completed 6 choice tasks in which they were asked to choose between experimentally-designed surgical options with varying levels of risk of nerve damage, hypocalcemia, need for a second surgery, need for daily thyroid medication, and cancer recurrence. Random-parameters logit models and resulting preference weight estimates were used to infer patients’ preferences for surgical profiles representing lobectomy and total thyroidectomy.

**Results:** From 2017-18, 150 adult patients requiring surgery for a low-risk thyroid cancer or thyroid nodule were enrolled. Median age was 58 years; 80% were female. Results suggested that patients on average favored total thyroidectomy largely due to an assumed 40% risk of requiring a second surgery after lobectomy. If the risk of needing a second surgery can be reduced to 31% or less, the average patient would favor lobectomy over total thyroidectomy.

**Conclusions:** Patients facing decisions about surgery for thyroid cancer will encounter varying levels of surgery-related risks due to tumor characteristics and experience of their surgeon. Preference assessments are essential to promoting shared decision-making for low-risk thyroid cancer. By characterizing patient preference, DCEs have the potential to improve patient satisfaction and outcomes, and shape regulation and health care policy.
**Extent of Surgery for Low Risk Thyroid Cancer in Elderly: Equipoise in Survival but Not in Short-Term Outcomes.**

Alan Zambeli-Ljepović, BS; Frances Wang, MS; Michaela A. Dinan, PhD; Terry Hyslop, PhD; Michael T. Stang, MD; Sanziana A. Roman, MD; Julie A. Sosa, MD, MA; and Randall P. Scheri, MD

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**Background**: Papillary thyroid cancer (PTC) is the fastest increasing cancer in the U.S. Despite an excellent prognosis for PTC overall, treatment has not been standardized for the elderly population, who are also more likely to suffer complications from thyroid surgery. Our aim in this study was to investigate the association between extent of surgery (total thyroidectomy vs. lobectomy) and complications, emergency department (ED) visits, and readmissions among older adults with low risk PTC. We hypothesized that all three outcomes are associated with total thyroidectomy.

**Methods**: The linked SEER-Medicare database was used to identify patients ≥66 years treated for T1N0M0 PTC between 1996 and 2011. Multivariable logistic regression was used to evaluate the adjusted effect of extent of surgery on the following: 30-day complications, endocrine-specific complications lasting ≥6 months, ED visits that did not result in hospital admission, and 90-day unplanned readmissions.

**Results**: A total of 3341 patients met inclusion criteria; 77.3% were female, mean age was 72.9 years, and mean tumor size was 0.8 cm. Overall, 67.6% underwent total thyroidectomy; 32.4% underwent lobectomy. On multivariable analysis, patients treated with total thyroidectomy were more likely to have 30-day complications [odds ratio (OR) 1.99, P < 0.01] and to be readmitted after surgery (OR 1.59, P < 0.01). 30-day complications were also independently associated with female sex (OR 1.34), black race (vs. white, OR 1.65), and comorbidity index (≥2 vs. 0, OR 1.43); all P < 0.01. Six-month endocrine complications were more common in female patients (OR 1.37, P = 0.03). Black patients and those with ≥2 comorbid conditions were more likely to present to the ED (OR 1.50 and 1.92, respectively) and to be readmitted after surgery (OR 2.19 and 2.29); all P < 0.01.

**Conclusions**: Despite equipoise in national operative recommendations, total thyroidectomy for older adults with low risk PTC is associated with potentially avoidable complications and readmissions, particularly among black and female patients. In many cases, lobectomy may be a safer and less costly alternative.
Behavioral Neurosciences Study Program (BSP)
Neurosciences Study Program (NSS)
Ophthalmology and Visual Science Study Program (OVS)
Infants who watch Television become Toddlers who watch more Television: Screen Time Patterns in the Greenlight Study

Alexander J. Hish, BS, Charles T. Wood, MD, MPH, Janna B. Howard, MPH, H. Shonna Yin, MD, MS, Russell L. Rothman, MD, MPP, Lee M. Sanders, MD, MPH, Alan M. Delamater, PhD, Kori B. Flower, MD, MS, MPH, Eliana M. Perrin, MD, MPH

Background: Little is known about patterns of television (TV) watching in infancy or whether infant television watching predicts two-year-old screen time. We aimed to determine if TV watching in the first 6 months of life is associated with TV watching at age 2 and whether this differs by sociodemographic factors.

Methods: We utilized longitudinal data from infants enrolled in the control group of Greenlight, a cluster randomized multi-site trial to prevent childhood obesity at pediatric resident clinics which included measures of time spent watching TV (“active” screen time) at well-child visits between 2 to 24 months. Caregivers were asked “On a typical day, how much time does [child’s first name] spend watching television?” and reported duration (average minutes/day). TV time minutes were dichotomized as none or any at each age based on AAP recommendations of 0 minutes for children younger than 18 to 24 months. We grouped children into four categories based on whether active TV time was introduced by 2, 4, or 6 months, or whether no TV time was endorsed at any of these timepoints. We measured the prevalence of children watching any TV at each well child check (WCC), performed chi-square analyses to measure associations among TV time patterns and sociodemographic factors, and performed a Kruskal-Wallis test comparing median TV time at 24 months between the 4 categories of TV watching.

Results: 235 caregivers had complete data for minutes of TV watched at 2, 4, and 6 months. Only 15.4% of infants did not watch TV at all from 2 to 6 months, while 33.6% were already watching by their 2 month WCC, another 20.4% started by their 4 month WCC, and an additional 8.9% started by their 6 month WCC. TV time at 24 months differed between groups ($p = .05$), with a median time (in minutes) of 60 (in the groups starting TV viewing at 2 months or at 4 months), 48 (in the group starting at 6 months), and 30 (in the no TV from 2-to-6 months group). Time of introduction in the first 6 months was significantly associated with African-American race ($p = .004$); Latino ethnicity ($p = .006$); being born in the U.S. ($p = .011$); and any TV viewing at 9 months ($p < .001$), 15 months ($p = .008$), and 18 months ($p = .001$).

Conclusions: A high percentage of infants start to watch television within the first two years of life and the amount of TV watching at age 2 is inversely related to age of introduction. Patterns of viewing as early as the first 6 months predict later viewing practices. While rates differ by race/ethnicity, TV watching in infancy across groups is a reminder to start earlier with universal anticipatory guidance about avoidance of screen time.
Multiple Sleep Dimensions and Chronic Kidney Disease: Findings from the Multi-Ethnic Study of Atherosclerosis

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Medical Student Research Fellowship
National Institutes of Health, National Institute of Environmental Sciences

Background: Poor sleep dimensions may contribute to chronic kidney disease (CKD) through hypoxia-induced systemic and intraglomerular pressure, inflammation, oxidative stress, and endothelial dysfunction. However, few studies have investigated the association between objectively-measured sleep dimensions and CKD, especially in a racially/ethnically-diverse US cohort.

Methods: We investigated the association between sleep dimensions and CKD among 1,895 Multi-ethnic Study of Atherosclerosis (MESA) Sleep Ancillary Study participants who completed in-home polysomnography, wrist actigraphy, and a sleep questionnaire. Using Poisson regression models with robust variance, we estimated separate prevalence ratios (PR) and 95% confidence intervals (CI) for CKD (glomerular filtration rate [GFR] <90 ml/min/1.73m², or albuminuria>30 mg/g) and moderate-to-severe CKD (GFR <60 ml/min/1.73m², or albuminuria>30 mg/g) among participants with versus without poor sleep dimensions (e.g., short (<7-hours) and very short (5-hours) sleep duration, apnea-hypopnea index (AHI) 3%, and Box-Cox transformed continuous values of sleep apnea-specific hypoxic burden (SASHB) [total area under the respiratory event-related desaturation curve divided by total sleep duration, %minute/hour]). We adjusted for sociodemographic characteristics and lifestyle/health behaviors.

Results: Of the 1,895 participants, mean age was 68.2±9.1 years, 54% were women, 37% were White, 28% Black, 24% Hispanic/Latino, and 11% Asian. Among participants with AHI ≥15 events/hour and in the highest quintile of SASHB, we observed suggestive positive associations with CKD (PR_AHI=1.04 [95% CI: 0.98-1.10], PR_SASHB=1.10 [0.99-1.21]) and moderate-to-severe CKD (PR_AHI=1.20 [0.97-1.48], PR_SASHB=1.37 [0.97-1.93]). Very short sleep duration (PR=1.36 [1.04-1.78]) and Box-Cox transformed SASHB (PR=1.07 [1.02-1.12]) were positively associated with a significantly higher prevalence of moderate-to-severe CKD.

Conclusions: Sleep dimensions related to hypoxia and short sleep, potentially representing independent biological mechanisms, were positively associated with a higher prevalence of CKD across multiple races/ethnicities in the US. Future prospective studies with larger samples and more cases are needed to further investigate the relationship between poor sleep dimensions and CKD.
Evaluating the contribution of mGluR5 signaling in striatal astrocytes to OCD-like behavior in the mouse model

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Eugene A. Stead, Jr. Student Research Scholarship

**Background:** The metabotropic glutamate receptor 5 (mGluR5) is a G-protein coupled receptor expressed throughout the central nervous system. Altered mGluR5 function has been associated with multiple neurological and psychiatric conditions, including autism-spectrum disorders. In mice, knockout of the post-synaptic density (PSD) scaffolding protein Sapap3 causes increased, constitutive mGluR5 signaling resulting in OCD-like behavior (Ade et al. *Biol. Psychiatry*, 2016). Constitutive mGluR5 signaling occurs when its interaction with the scaffold protein Homer is disrupted (Ango et al. *Nature*, 2001). In disorders with abnormal mGluR5 scaffolds, the cell or cells in which mGluR5 signaling drives abnormal behavior remain unknown. Astrocytes are compelling non-neuronal candidates. They express mGluR5 and PSD scaffolding proteins, especially early in postnatal development (Petrelli et al. *Curr. Opin. Neurobiol.*, 2018), and reducing astrocyte calcium signaling in the dorsolateral striatum (DLS) is sufficient to induce repetitive grooming behaviors (Yu et al. *Neuron*, 2018). Since mGluR5 signals via Gq G-proteins to release calcium, these observations suggest that mGluR5 signaling in striatal astrocytes could contribute to OCD-like behavior.

**Methods:** To induce constitutive mGluR5 signaling in striatal astrocytes, I used Cre-recombinase conditional adeno-associated viral constructs expressing a short, HA-tagged peptide containing the mGluR5 C-terminus Homer binding site (mGluR5CT), previously shown to disrupt mGluR5-Homer binding, or a scrambled mutant control peptide (mGluR5MU) (Mao et al. *J Neurosci.*, 2005). Viruses were delivered via stereotaxic injections targeting the DLS in adult (P41-P113) mice expressing an astrocyte-specific, tamoxifen-inducible Cre (Aldh1L1-creERT2), followed by 5 days of tamoxifen injection to induce peptide expression. Mice were evaluated for locomotor, anxiety-like, and grooming behaviors after 4-6 weeks. Viral spread was documented post-mortem using serial sections of fresh-fixed brain tissue.

**Results:** Behavioral evaluation revealed sexual dimorphism in locomotor behavior induced by astrocyte mGluR5CT peptide, with more consistent differences in males. Males with disrupted astrocyte mGluR5-Homer binding (n=11) displayed decreased locomotion in the open field test (two-way rmANOVA, virus effect on distance traveled per 5-min interval, F$_{1,19}$=12.09, p=0.0025, Sidak’s correction) and a trend toward decreased locomotion in the elevated zero maze (715.6 cm vs. 871.7 cm, p=0.097, Welch’s t-test) compared to controls (n=10), without coordination or strength deficits. Astrocyte mGluR5-Homer disruption induced non-statistically significant trends toward increased anxiety-like behavior. Post-mortem evaluation of viral spread demonstrated expression of mGluR5CT/MU peptides in multiple brain regions, including the corpus callosum, striatum, and thalamus.

**Conclusion:** Disruption of astrocyte mGluR5-Homer interactions results in decreased locomotion and trends toward anxiety-like behavior in male mice. Because viral expression was more widespread than intended, future studies are needed to document the specific contribution of striatal astrocytes. However, these data demonstrate that manipulating mGluR5-Homer scaffolds in astrocytes alone has the potential to alter behavior.
TOTAL SERUM LEVELS OF IMMUNOGLOBULIN A IN AMYOTROPHIC LATERAL SCLEROSIS

Crayle J, Bedlack R.

Background: Amyotrophic lateral sclerosis (ALS) is a clinically heterogenous motor neuron disease without an identifiable etiology in 85% of cases. Described pathophysiologic features include neuroinflammation, lower regulatory T-cell levels, and possibly an altered fecal microbiome. All of these pathophysiologic features can affect total serum immunoglobulin A (IgA) levels. Therefore, we hypothesized that IgA levels are altered in ALS.

Methods: A database of the electronic health record at a tertiary referral academic medical center was used to extract data from the charts of patients with ALS-spectrum disease, other neurodegenerative diseases, select chronic autoimmune neurologic diseases, and those neurologically healthy.

Results: Initial analyses demonstrated over one-fifth of study subjects with ALS had an IgA level above the laboratory reference range at our institution and also suggested that patients with ALS have significantly higher IgA levels relative to neurologically healthy controls. This difference however disappeared when controlling for age and sex and it was determined that over 20% of neurologically healthy subjects also have elevated IgA levels per the reference range. A possible association of ALS clinical disease features with IgA levels was also explored. In addition to these findings, there were several unexpected observations including a possible association of dementia with higher IgA levels and a possible association of myasthenia gravis with lower IgA levels. Of these two associations, the association of myasthenia gravis was more robust to subsequent analyses to control for extraneous variables. We speculate that this association is either due to regulatory T-cell and B-cell dysfunction in the context of myasthenia gravis or immunosuppressive therapy.

Conclusions: We found no association between IgA levels and ALS suggesting a revision of the laboratory reference range would be appropriate. We did find several possible associations of IgA levels with neurologic disease that could be explored in future research.
Impact of Early Palliative Care for Patients with Brain and Spine Metastases

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Background: Metastases to the brain or spine represent particularly devastating sequelae of a cancer diagnosis, resulting in poor patient survival, function, and health-related quality of life. Brain and spine metastasis patients are faced with complicated decision-making balancing intervention options, end-of-life planning, and a high overall symptom burden. Palliative Care (PC) has been shown to provide supportive services to patients at the end of life, while providing guidance on symptom management, end-of-life planning, and complex communication between patients, caregivers, and providers. Despite the benefits of Palliative Care consultation, this resource remains infrequently consulted within the context of central nervous system metastases. No prior study has formally evaluated the rates of PC referral in this population, or the impact of specialty referral within this context. Palliative Care may ultimately be an effective intervention to improve health-related quality of life for these patients, as has been robustly shown in other disease groups.

Methods: Analysis was performed of the utilization rates of Palliative Care consults for both brain and spine metastases patients in inpatients admitted to Duke University Medical Center, Duke Regional Hospital, and Duke Raleigh Hospital. Consult rates were analyzed according to hospital site as well as discharging service. Data was obtained from FY16 through December of FY19. Brain and spine metastases patients were captured using ICD-9 and -10 codes corresponding to “brain metastases” and “bone metastases,” respectively. Palliative Care utilization rates captured all consults to the Palliative Care service placed while a brain or spine metastasis patient was admitted inpatient, including both in-hospital consults with the Palliative Care team and/or referral to outpatient Palliative Care follow-up. Outpatient Palliative Care referrals made after discharge were not captured by this analysis.

Results: A total of 2608 discharges were analyzed (2397 brain metastasis discharges, 301 spine metastasis discharges). The average number of inpatient brain and spine metastasis patients receiving a Palliative Care consult was 13.6% and 11.0% over this 3.5 year period, respectively. Duke University Hospital had the lowest utilization – with 6.4% of brain and 7.6% of spine metastasis patients receiving Palliative Care consults – as compared to Duke Raleigh Hospital and Duke Regional Hospital. For both brain and spine cohorts, Neurology was the discharging service with the highest rates of PC utilization, consulting Palliative Care for 37.7% of brain and 42.9% of spine metastasis patients respectively. Surgery, Neurosurgery, and Medicine had the lowest rates of Palliative Care consult utilization. Over time, PC utilization increased for brain, but not spine, metastasis patients.

Conclusions: Palliative Care consultation rates remain low amongst patients with brain and spine metastases, despite the high symptom burden and low overall survival for many of these patients. Ultimately, these single-institution utilization patterns may provide a microcosm for national patterns and trends at similar institutions. Utilization trends suggest that although PC consultation rates may be increasing over time in certain patient populations, rates of referral remain persistently low in other populations. Future work should focus on elucidating provider- and patient-specific barriers to Palliative Care referral as well as analyzing the impact of specialty Palliative services on patient health-related quality of life and clinical outcomes.
Healthcare Resource Utilization and Management of Chronic, Refractory Low Back Pain: A Retrospective Analysis of Health Insurance Claims Data

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Background: The total economic burden of low back pain (LBP) in the US is estimated between $84.1 and $624.8 billion. Some patients with persistent LBP despite conventional medical management are ineligible for spine surgery and are considered to have chronic, refractory low back pain (CRLBP). We investigated the healthcare resource utilization of this population.

Methods: The IBM MarketScan® Research databases were used to identify adult patients with a diagnosis of non-specific LBP, including spondylosis without myelopathy or radiculopathy, degenerative disc disease, and stenosis without claudication. We excluded patients with history of cancer, spine surgery, or failed back surgery syndrome within the study period (2009-2016) or pregnancy in the year prior to diagnosis. For patients to qualify as refractory, we required >30 days of utilization of pain medications (prescribed within 2 weeks of diagnosis) or non-pharmacologic therapies within both the 3-12- and 12-24-month periods post-diagnosis. Healthcare costs and the frequency of utilization of prescribed pain medications and other therapies were calculated for 2 years.

Results: Among 50,801 patients, median total cost was $3,755 (IQR $1,299–$9,108) for the 1 year pre-diagnosis, reached $6,622 (IQR $2,723–$13,978) in the first year, and decreased to $5,977 (IQR $2,311–$13,307) in the second year. Outpatient services accounted for the majority of all costs. Costs were highest for patients with Medicare. Of the entire cohort of 55,945 patients, over 40% engaged chiropractors or PT, and >13% of patients used epidural or facet steroid injections. On average, 49.1% of patients used prescription pain medications, most commonly opioids (40.5%), muscle relaxants (21.5%), and anticonvulsants (17.8%).

Conclusions: This study was the first to our knowledge to investigate the healthcare costs and patterns of utilization of prescription medications and non-pharmacologic therapies within the first 2 years of a CRLBP diagnosis. For patients with CRLBP, the median annual total cost at 1 year almost doubled the baseline cost and decreased for the second year; most costs were due to outpatient services. Patients with Medicare incurred the highest total costs. The most-utilized therapies were chiropractic therapy, physical therapy (PT), and steroid injections. Opioids, muscle relaxants, and anticonvulsants were the most common prescription medications. Such data will help inform decisions about the management of CRLBP.
Caveolin-mediated mechanosensation in Schlemm’s canal endothelia

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Research to Prevent Blindness Medical Student Eye Research Fellowship

Background: Caveolae are specialized membrane structures that respond to mechanical perturbations by protein component disassembly and releasing molecules such as endothelial nitric oxide synthase (eNOS). Our laboratory has previously shown that mechanical stimulation of primary human Schlemm’s canal (SC) cells induces phosphorylation of CAV1 and eNOS activation and NO production; however, this mechanism of activation is unknown. We hypothesize that caveolae are the mechanosensors that link biomechanical stimulation of SC to eNOS activation, and that caveola-dependent eNOS activation enhances conventional outflow.

Methods: To study the effect of Cav-1 silencing in SC cells, we tested a novel Cav-1 shRNA silencing adenovirus with GFP tag (Vector Biolabs) and Scramble shRNA with GFP tag (control virus) in primary human Schlemm’s canal cells extracted and cultured in our laboratory, and in Huvec cells (Clonetics). GFP signal from infected and control cells was detected via flow cytometry at 72 hours for Schlemm’s canal cells and at 60 hours for Huvec cells to determine transduction efficiency. Total Cav-1 protein expression was also monitored by Western blotting using total Cav-1 and GFP antibodies. Western blot densitometry analyses were done using Image Lab software.

Results: We have found that the transduction efficiency of Huvec cells is 98% at a multiplicity of infection (MOI) of 10 with the Cav-1 shRNA silencing adenovirus, while that of Schlemm’s canal cells is 78% at an MOI of 50. Total Cav-1 protein whole protein analysis by western blot showed total Cav-1 protein expression in Schlemm’s canal cells infected with Cav-1 shRNA silencing adenovirus at MOI of 50 was decreased to 51% when compared to control cells, while that of Huvec cells was decreased to 30%.

Conclusion: We have effectively used Cav-1 shRNA GFP tagged adenovirus to achieve targeted knock down of Cav-1, which is the primary scaffolding protein of caveolae in human Schlemm’s canal cells. This lays a solid foundation to further study the role of Cav-1 knock down in eNOS activation and NO production via presence and absence of stretch and shear stress in primary human SC cells.
Impact and Implication of Fovea-Involving Intraretinal Hemorrhage Following Acute Branch Retinal Vein Occlusion

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Backgrounds: Retinal vein occlusion is the second most common retinal vascular cause of vision loss after diabetic retinopathy, with branch retinal vein occlusion (BRVO) having an incidence of 0.5 to 1.2% in the United States. Certain funduscopic findings have been postulated to be associated with a worse visual prognosis, such as the presence of macular ischemia and macular edema, while other findings are of uncertain significance, such as fovea-involving intraretinal hemorrhage (IRH). The purpose of this project is to compare clinical outcomes in patients with acute branch retinal vein occlusion (BRVO) with (group A) or without (group B) fovea-involving IRH.

Methods: Retrospective study utilizing a database of patients diagnosed with acute, treatment-naïve BRVO over an 8-year period. The presence of fovea-involving IRHs was determined from baseline fundus photographs by human graders and was confirmed with multimodal imaging. Presenting features, treatment patterns, and clinical outcomes were compared.

Results: Of 172 patients with BRVO, 33 (19.2%) had fovea-involving IRHs at presentation. Corrected logMAR VA was significantly worse in group A than in group B at baseline (0.54 ±0.06 [20/69] vs. 0.34±0.03 [20/44], p=0.001). Group A was also more likely to have CME (93.9% vs. 48.2%, p<0.001) and greater CST at baseline (523.8±32 vs. 345.9±11.8 µm, p<0.001) and required more anti-VEGF injections in the first year (4.50+3.43 vs. 1.89+3.26, p<0.001). Final logMAR VA was also worse in group A (0.57±0.12 [20/74] vs. 0.35±0.05 [20/45], p=0.05); a significantly greater number of patients in group A had decreased in vision of ≥2 (36.4% vs. 18.7%, p=0.04) or ≥3 lines (27.3% vs. 10.8%, p=0.05) at the final follow-up visit. Although patients in group A had a higher prevalence of CME (63.6% vs. 27.3%, p<0.001) at the final follow-up visit with greater treatment burden, these patients experienced a significantly greater decrease in CST (-197.8±45.3 vs. -51.7±14.7µm, p=0.005).

Conclusions: Acute BRVO presenting with fovea-involving IRHs is associated with significantly worse presenting features, greater treatment burden, and worse clinical outcomes despite current therapeutic interventions.
Epidemiology and Public Health Study Program (EPH)
Global Health Study Program (GHSP)
Dual Degree Program (MBA)
Medical Humanities Study Program (MEDHUM)
Primary Care Leadership Track (PCLT)
A Comparison of Racial and Ethnic Representation in Clinical Trials and Registries of Heart Failure

Oludamilola Aladesanmi, MPH, Godefroy Chery, MD, Larry R. Jackson II, MD, Kevin Thomas, MD

Background: Clinical trials of heart failure produce recommendations that inform guidelines for practitioners, but underrepresented racial and ethnic groups (UREGs) remain underrepresented in these trials. Lack of UREG engagement in these trials limits the generalizability of their results, which could contribute to significant differences in health outcomes among UREGs at risk of heart failure. Registries of heart failure patients have the potential to be more representative of the population due to their more lenient inclusion and exclusion criteria as well as their observational rather than interventional nature. Little is known about the reporting and representation of UREGs in clinical trials compared to registries of heart failure. We conducted a meta-analysis to compare the relative proportion of UREGs in clinical trials versus registries of heart failure. We hypothesized that registries of heart failure would have a relatively higher proportion of UREGs compared to clinical trials because of their less strict inclusion and exclusion criteria and their observational nature.

Methods: We searched PubMed and Embase for randomized controlled trials (RCTs) and registries of heart failure published from 2010-2019. All included studies recruited patients from North America only and comprised human subjects greater than 18 years of age. We excluded non-randomized trials, non-registries, and secondary analyses, as well as studies recruiting from outside North America. A total of 1424 articles were identified and subsequently screened by two reviewers. After screening, we abstracted study title, NCT number (if applicable), first author, year of publication, location, race, ethnicity, age, sex, inclusion and exclusion criteria, journal, sample size, intervention, study population, funding source, recruitment setting, and outcomes. Secondary information was supplemented by Clinicaltrials.gov and/or publication of primary results, if applicable. We compared the relative proportion of different races and ethnicities in clinical trials and registries of heart failure.

Results: Our review yielded 86 clinical trials, enrolling a total of 20,764 patients, and 12 registries of heart failure, enrolling a total of 223,757 patients. 78% of clinical trials (n = 67) reported information on race compared with 83% (n = 10) of registries (p = 0.668). Of the studies that reported race, 69% of trials (n = 45) and 50% of registries (n = 5) reported discrete racial and ethnic groups as opposed to white versus nonwhite (p = 0.230). We found that Black patients were less likely than White patients to be enrolled in HF registries than clinical trials (ORs 0.216 vs. 0.0313, p = 7.70 x 10^-4).

Conclusions: Our analysis showed significant differences in the relative enrollment of different races and ethnicities in clinical trials versus registries of heart failure in our analysis. Given the evidence of racial and ethnic disparities in heart failure as well as the increasing demographic diversity within North America, we recommend future studies and an updated systematic review in the future. We specifically recommend similar future investigations for other cardiovascular diseases, given the disparate impact of cardiovascular disease on UREGs as well as the high burden both in the US and abroad.
Apnea in Pre-Term Infants Following Vaccination: A Systematic Review of the Literature

Bryanna Carpenter, Chip Walter

**Background:** Vaccines administered during the first year of a child’s life are crucial for preventing multiple diseases. Concerns about vaccine safety in premature infants including the perceived risk of apnea after immunization likely contributes to underimmunization in this population. This paper conducted a systematic review of the literature to assess cardiorespiratory events (CREs) following administration of vaccines in preterm infants.

**Methods:** PubMed, Embase, Cochrane, Scopus, and Clinicaltrials.gov were searched starting in 1970, or the earliest dates available for the database, and going through October 18, 2018. Data was extracted using a standardized extraction tool, and quality was assessed using Cochrane and NIH guidelines.

**Results:** 516 non duplicated articles were found. 433 articles were excluded after title and abstract screen, leaving 84 articles for full article review. Of these 84 articles, 31 were included in the review. There were a total of 15 prospective cohort studies, 14 retrospective cohort studies, and 2 randomized controlled trials. Results of the review showed mixed results. Overall, 19 studies showed a statistically significant difference in CREs post vaccination, while 13 showed no difference.

**Conclusions:** Overall, incidence of cardiorespiratory events vary widely between papers. Despite there being many studies that have looked at apnea in pre-term infants after vaccination, there is not enough evidence either way to recommend an action on vaccinations in preterm infants. More, large scale RCTs should be conducted to make a conclusive recommendation.
Diabetes in women with early-stage breast cancer receiving chemotherapy at a tertiary medical center.

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Background: Women with diabetes who develop breast cancer have greater all-cause and cancer-specific mortality than women without diabetes; however, it is unclear whether this disparity is due to diabetes, differential breast cancer treatment, or factors associated with both breast cancer and diabetes, such as obesity or sociodemographic differences. We evaluated these characteristics among a sample of women with early-stage breast cancer receiving chemotherapy at a tertiary medical center in the southeastern United States where diabetes is prevalent. The goal of this study was to assess differences in mortality and to compare health characteristics between women with early-stage breast cancer with and without diabetes.

Methods: A retrospective chart review of women receiving chemotherapy for early-stage breast cancer who first visited the Duke University Cancer Institute between January 2015 to July 2017. Data included sociodemographic variables, alcohol and tobacco use, cardiovascular disease, cancer treatment (radiotherapy, surgery, steroid use) and health care utilization. Diabetes was defined by ICD code or a HgbA1c of ≥6.5% (48 mmol/mol).

Results: A total of 223 patients met study criteria and were included in this analysis. Women with diabetes tended to be older and made up 31.8% of the cohort with a median HgbA1c of 7.2% (55 mmol/mol). Women with diabetes more often had coronary artery disease and stroke. The median follow-up time from first clinical visit was 2.9 years (range 0.2 - 5.5). There were no statistically significant differences between patients with and without diabetes in demographics, cancer treatment or health care utilization. Since only 6 deaths were reported, predicting overall survival was not possible.

Conclusions: Our findings showed a higher prevalence of diabetes in patients with breast cancer than expected based on previous studies. Due to the short follow-up time and few deaths among this cohort, a hazard model of mortality was not possible. However, given the increasing incidence and prevalence of diabetes in the United States and known association of diabetes with poor general health and breast cancer-specific outcomes, more study is warranted among women with early-stage breast cancer and diabetes.
Neurodevelopmental Outcomes for Children Born Prior to 28 Weeks Gestation: Relationship to Indication for Preterm Delivery

Ariana Stewart, MPH, Julie Daniels, PhD, and Michael O'Shea, MD, MPH

Background: Individuals born extremely preterm (prior to 28 weeks’ gestation) are at high risk for a variety of neonatal complications, including respiratory distress syndrome (RDS), sepsis, chronic lung disease, and brain injuries, as well as long-term neurodevelopmental impairments, such as intellectual deficit (IQ), low academic performance, cerebral palsy, autism spectrum disorder (ASD), attention-deficit hyperactivity disorder (ADHD), and anxiety. This variety of poor outcomes makes this unique population important to study in the field of public health. This study explores the relationship between the indication for extremely preterm delivery and neurodevelopmental outcomes in the offspring at 10 years of age.

Methods: Data were obtained from the Extremely Low Gestational Age Newborn (ELGAN) multicenter cohort of 889 children born prior to 28 weeks’ gestation from 2002 - 2004. Based on prior research on this cohort, the indications for extremely preterm delivery were classified as having underlying intrauterine inflammation (preterm premature rupture of membranes (PPROM), placental abruption, cervical insufficiency, or idiopathic preterm labor), or placental dysfunction (preeclampsia or fetal indication/intrauterine growth restriction). Cognitive impairment, autism spectrum disorder (ASD), epilepsy, and cerebral palsy (CP) were identified by in-person evaluation of study participants at 10 years of age. Odds ratios (OR) and 95% confidence intervals (CI) were estimated using logistic regression.

Results: While the majority of the children in each indication group had no cognitive impairment, the intrauterine inflammation group had a slightly lower percent of no impairment compared to placental dysfunction group. Based on broad groupings of the indications for preterm delivery, children delivered as a result of placental dysfunction group had 2.35 times the odds of development of mild cognitive impairment compared to those born due to idiopathic preterm labor (95% CI 1.30 – 4.24). The majority of the cohort did not have any neurodevelopmental impairments and development of ASD, CP and epilepsy did not vary by indication for preterm delivery. The one exception was the increased odds of CP among children born following preterm delivery indicated by cervical insufficiency, though the estimate was imprecise.

Conclusions: The aim of this study was to explore the relationship of indications for preterm delivery and neurodevelopmental outcomes at 10 years of age for those born extremely preterm. Our most important finding was that fetal indication for preterm delivery was associated with a higher risk of mild cognitive impairment than other indications of delivery. Despite the different etiologies leading to the early delivery, this study highlights relatively similar outcomes at 10 years of age. Most children do not have long term adverse outcomes and most outcomes that did arise were not different by preterm indication. Despite this, those born extremely preterm, including this cohort, have poorer outcomes than the general population.
**Network Characteristics of a Hypertensive Referral System in Western Kenya**


*Doris Duke Charitable Foundation International Clinical Research Fellowship*

**Background:** The Strengthening Referral Networks for Management of Hypertension Across the Health System (STRENGTHS) trial is creating and testing interventions to improve the effectiveness of referral networks for patients with hypertension in Western Kenya. Network analysis of facility-based healthcare providers was used to understand the existing pattern of referrals. The ultimate goal was to quantify network structures and identify both structural gaps and opportunities for implementation of the planned intervention.

**Methods:** A network survey was administered to providers who care for patients with hypertension asking individuals to nominate a) individuals to whom, and b) facilities to which they refer patients, both up and down the health system. We analyzed measures of in-degree and out-degree (number of links each provider received and sent, respectively), and logistic regression to identify predictors of referral patterns. We measured strength of the network with a core-periphery (CP) model, whereby higher CP indicates a strong referral network.

**Results:** We surveyed 130 providers across 39 sites within 7 geographically separate clusters. Individuals were more likely to refer patients to providers who were male, had a higher job title, had more years working, and were based at a higher facility level. Fifty-one providers (39%) stated that they do not refer patients down, while three providers (2%) do not refer up. Compared to a perfect CP referral network model (CP = 1.00), the provider referral networks within each cluster showed a weak CP structure (CP range 0.33 to 0.64). In contrast, cluster-level facility networks showed a strong tendency for CP structure, with a CP range of 0.86 to 0.95.

**Conclusions:** This hypertensive referral system demonstrates opportunities for improvement. First, up-referrals are more common than down-referrals and provider characteristics impact the likelihood of receiving a referral. Secondly, while facility-to-facility referrals are closer to a perfect referral model, there are gaps in communication between the specific providers. There is need for STRENGTHS to design and test interventions that strengthen provider referral patterns and balance referrals both up and down the system in order to improve blood pressure control and reduce cardiovascular risk.
Incidence of acute Q fever and spotted fever group rickettsioses, Kilimanjaro, Tanzania, 2007-2008 and 2012-2014

Doris Duke International Clinical Research Fellowship

Background: Q fever and spotted fever group rickettsioses (SFGR) are common zoonotic causes of non-malarial febrile illness and can cause substantial morbidity. Both diseases often have nonspecific presentations, such as fever, myalgia, headache, and fatigue. Accurate diagnosis is difficult, particularly in resource-limited areas where appropriate diagnostic testing is unavailable. Q fever and SFGR often cause severe febrile illness in northern Tanzania; however, under-recognition and under-reporting of cases make estimating disease incidence a challenge. While disease prevalence data can inform patient management, incidence estimates are needed to characterize disease burden and prioritize disease prevention measures and public health policies. To our knowledge, there are currently no incidence estimates for either Q fever or SFGR in sub-Saharan Africa. We aimed to provide the first incidence estimates of these diseases for northern Tanzania.

Methods: Using sentinel surveillance at two referral hospitals, we estimated the incidence of Q fever and SFGR in Moshi Municipal and Moshi Rural Districts in the Kilimanjaro Region of Tanzania during the periods 2007-2008 and 2012-2014. Paired serum samples were obtained from febrile patients at enrollment and 4-6 weeks later. Confirmed acute cases were defined as a ≥ fourfold increase in antibody titer between paired samples by indirect immunofluorescence assay (IFA) to C. burnetii phase II antigen for acute Q fever and R. conorii (2007-2008) or R. africae (2012-2014) antigens for SFGR. We used a healthcare utilization survey to adjust for under-ascertainment of cases by sentinel surveillance. Uncertainty ranges for incidence point estimates were derived using 95% confidence intervals (CI) for observed prevalences and healthcare-seeking adjustment multipliers, as well as plausible sensitivity and specificity ranges of paired sera IFA for both diseases.

Results: For 2007-2008, among 557 febrile participants from the catchment, 17 (5.3%) of 322 and 24 (8.2%) of 292 with paired sera had confirmed Q fever and SFGR, respectively. Adjusted incidence estimates (uncertainty range) of Q fever and SFGR were 89 (23-474) and 106 (41-525) per 100,000 persons annually, respectively. For 2012-2014, among 1,114 febrile participants from the catchment, 50 (7.8%) and 55 (8.6%) of 641 with paired sera had confirmed Q fever and SFGR, respectively. Adjusted incidence estimates (uncertainty range) of Q fever and SFGR were 56 (24-158) and 65 (30-151) per 100,000 persons annually, respectively.

Conclusion: We found a moderate incidence of both diseases during both study periods in northern Tanzania. To our knowledge, these are the first estimates of Q fever and SFGR incidence in sub-Saharan Africa. Our findings suggest that prevention and control measures for these infections warrant further consideration.
**Effect of Fragmented Oncologic Care for High-Risk Pediatric Neuroblastoma Patients – A Single Institution Analysis**

Aaron Tarnasky, BS, Meredith Achey BA, Luke Wachsmuth, BS, Hannah Willamson, MS, Elisabeth Tracy, MD, Timothy Driscoll, MD

**Background:** Children with high-risk neuroblastoma (NBL) have high-mortality rates and undergo complex, multi-modal therapy. They may be subject to fragmented care among different providers and institutions, which has been associated with poor clinical outcomes for various adult cancers. These patients may also experience significant travel burden in accessing appropriately equipped facilities. We hypothesize that fragmented care for pediatric NBL patients would confer inferior outcomes compared to treatment consolidated at one location.

**Methods:** Both paper and electronic records for pediatric NBL patients having received ≥1 bone marrow transplant at our institution from 1990-2018 were manually reviewed. Variables collected include demographics, diagnostic and treatment characteristics, complications, and relapse and survival outcomes. Fragmented care is defined by treatment occurring at >1 institution and grouped according to 2 institutions vs. >2 institutions. Distances to treatment were calculated as the driving distance between a patient’s home postal code and that of the treating hospital using Google Maps. Fisher’s exact and Kruskal-Wallis tests were used to compare patients receiving fragmented versus consolidated care. Unadjusted overall survival (OS) was estimated using the Kaplan-Meier method, and differences in OS between groups were tested using the log-rank test. Significant predictors of OS and relapse were calculated using multivariable Cox proportional hazards models.

**Results:** We extracted data from 128/148 patients. The most common reasons for exclusion included incomplete medical records (n=9) and BMT for relapse/recurrence only (n=8). 103 patients experienced fragmented care, with 18 of them being treated at 3+ facilities. More patients with consolidated care were above the 75th percentile for distance traveled to chemotherapy and surgery, while more patients with 3+ facilities were above the 75th percentile for distance traveled to BMT and immunotherapy (all p<0.01). On univariate analysis, neither fragmented care group was associated with mortality (both p’s>0.05). Diagnosis in earlier decades, particular chemotherapy protocols, enrollment on a clinical trial (compared to being treated according to its guidelines), and increased distance to BMT were all significantly associated with increased mortality (all p’s<0.05). Only diagnostic year and distance to BMT remained significantly associated with OS on multivariate analysis.

**Conclusion:** Our institutional analysis of high-risk NBL patients demonstrates no significant difference in OS or relapse rates based on degree of fragmented care or travel distance. These findings may be critical to those living far removed from appropriate treatment. Further research and interventions should explore lower-risk disease and aim to improve supportive processes for patients undergoing complicated and burdensome care.
Time-Driven Activity-Based Costing of Emergency Department Post-Discharge Nurse Calls

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Background: Post-discharge nurse calls can decrease patient representation rates to healthcare systems. Call program costs have not previously been compared to patient representation rates to determine cost-effectiveness. We used time-driven activity-based costing to determine the costs with such programs.

Methods: We developed process maps for a post-discharge nurse call program in the Emergency Department (ED) of an urban, quaternary care academic Level 1 trauma center. Our primary outcome was total cost of calls calculated from the length of calls after 8 hours of observation and total capacity rate costs from national nurse salary and space costs. We performed a retrospective analysis of 7-day representation rate differences of patients reached and not reached from July, 2018 to March, 2019 with a Z-test.

Results: We observed 113 post-discharge calls for 79 patients. Length of call for patients reached was 4.3 minutes (Standard Deviation [SD] 1.8) compared to not reached of 2.6 minutes (SD 0.6). The total capacity rate cost for calls was $1.09/minute, or $4.69 per patient reached and $2.83 per patient not reached. Retrospective analysis of 5,258 patients reached and 7,958 patients not reached showed representation rates of 2.4% and 4.2% (p<0.0001), respectively.

Conclusion: In this study, post-discharge nurse calls were associated with decreased representation rates to the ED and a cost of $448.33 to prevent one representation. However, costs saved from decreased representation, calculated as $378.03 per presentation to the ED from weighted reimbursement rates, may not offset program administration costs. Healthcare systems should also consider potential additional benefits of greater patient satisfaction and increased treatment adherence in determining use of post-discharge call programs.
Implementation of an EHR-based risk-stratified prostate cancer screening program in a primary care network

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Background: Prostate cancer screening recommendations and the proportion of men undergoing screening with a prostate specific antigen (PSA) test varies widely across the U.S., due largely to controversies regarding the harms and benefits of screening. As a consequence, screening rates are decreasing while the incidence of advanced stage prostate cancer is increasing, particularly among African American men. A multi-disciplinary group at Duke Health developed an age- and race-specific, risk-stratified screening algorithm, combined with shared decision making, for PSA follow-up testing. Our overarching goals were to determine implementation efficacy and identify clinically significant cancer while minimizing treatment of indolent cancer through a standardized practice model.

Methods: We evaluated the impact of implementing this risk-stratified screening algorithm in February 2017 in the health maintenance section of our health system-wide EHR (electronic health record; Epic). We used a pre-post implementation study design, comparing the percent of men who met up-to-date screening criteria, based on the algorithm, from 2/1/16-2/1/17 (pre-implementation) to 2/2/17–2/21/18 (post-implementation). Men were defined as ‘met screening’ if they had a PSA value recorded in our EHR within 27 months prior to their visit with their primary care provider (PCP). Descriptive statistics were performed followed by a one-sample pre-post test for binomial proportion and a two-proportion z test.

Results: During the pre- and post-implementation periods, 49,058 and 49,988 men, respectively, aged 40-74 were seen by 290 PCPs across 26 clinics (20.6% African American, pre and post). Overall, the percent of men who met screening criteria based on the algorithm increased from 48.9% (pre) to 67.5% (post) (p<0.0001); for African Americans it increased from 54% (pre) to 71.5% (post). The percent of men who met screening criteria increased across all age groups (p<0.001): age 40-45, 17 to 46%; age 45-49, 31 to 55%; age 50-69, 60 to 73.8%; and age 70-74, 51 to 67%. Similarly, the percent of men who met screening criteria increased across all clinic sites and all PCPs. Importantly, while screening according to the algorithm increased, the volume of PSA test ordering did not change. In the pre- and post-implementation period, 27,100 and 27,500 PSAs were ordered, respectively. This stability in PSA volume was largely due to a decrease in annual testing among older men in the post-implementation period.

Conclusions: With the implementation of an EHR-based risk-stratified algorithm, we were able to impact physician behavior, despite controversy around PSA screening, to observe a significant increase in meeting our screening criteria while avoiding a net increase in PSA test volume. This approach was successful in standardizing our network-wide approach to prostate cancer screening. Future efforts will evaluate costs and downstream clinical outcomes of this strategy.
Dying to Save: Conflicts of Interest Between For-Profit Serious Illness Care Organizations and Medicare Advantage Insurers

Ishaq Winters, Peter Ubel, M.D., Monica Lemmon M.D.

**Background:** There is a large push to contain health care costs in the U.S. Individuals who receive end of life care consume disproportionate resources and often receive poor quality care. Palliative care has the potential to increase quality and reduce cost of health care for individuals suffering from serious illness. However, access to palliative care is limited. In response, several for-profit companies that offer outpatient palliative care are emerging. These companies often partner with Medicare Advantage insurers to reach elderly beneficiaries. However, financial conflicts of interest that arise from these partnerships—such as the Medicare Hospice Benefit, value-based payment models, and for-profit ownership status—threaten to undermine their services. These conflicts of interest motivate Medicare Advantage insurers and for-profit palliative care organizations to encourage individuals to enroll in hospice and forgo costly intensive treatments. In this study, we evaluate the ethical implications of these financial conflicts of interest on outpatient palliative care and propose safeguards against this behavior to ensure that patients receive care according to their best interests.

**Methods:** We used Faden and Beauchamp’s “Three Conditions of Autonomous Action” as a theoretical framework for our ethical analysis of financial conflicts of interest on patient autonomy.

**Results:** Financial incentives motivate these companies to infringe on patient autonomy by unduly influencing beneficiaries to pursue hospice. Patients are susceptible to undue influence during advance care planning since this process is often their introduction to conversations about hospice. Undue influence may appear in various forms during these discussions. Financially motivated providers can restrict patient autonomy by manipulating the presentation of information, withholding less profitable treatment options, or coercing individuals to pursue hospice. Each of these potential forms of influence are unethical since they fail to fully inform the patient of their options and limit their ability to autonomously decide according to their preferences.

**Conclusions:** The health care community should establish safeguards to mitigate the risks of financial conflicts of interest within outpatient serious illness care. Stakeholders should first establish appropriateness criteria to gauge the behavior of these organizations. Additionally, public reporting of these appropriateness measures is essential for regulation of these partnerships. Finally, establishing a certification or accreditation process by an independent oversight body will enhance accountability and incentivize these companies to behave ethically.
Addressing Spiritual Concerns in the Emergency Department

Jennifer M. Frush, Farr A. Curlin, MD

**Background:** The role of spirituality in the healthcare setting has gained traction as a topic of interest to practitioners and researchers alike, but there has been little exploration in regards to spirituality in the emergency department (ED) specifically. The ED is unique in its acuity of care, spectrum of illnesses seen, brevity of clinician-patient interaction, and breadth of patients served.

**Methods/Results:** As a participant in the Medical Humanities Track, I took courses at the Duke Divinity School in the Theology, Medicine, and Culture (TMC) Fellowship. Through coursework, conversation, and multiple literature reviews, I realized there is a paucity of research on the topic of spiritual concerns of patients in the Emergency Department (ED). I developed a hypothesis that patients who present to the ED may have spiritual concerns that are unrecognized and/or not fully addressed by ED practitioners for a variety of reasons, such as time constraints or discomfort of the practitioner in broaching the topic. From dialogue with TMC scholars and EM faculty at Duke and beyond, we developed five representative clinical vignettes that are considered relatively common in the ED setting through which to explore the implications of how spiritual imagination and discourse may allow for deepening of the clinician-patient relationship and ultimately improve the delivery of health care. Drawing on issues raised through these vignettes, we presented practical heuristics that could be utilized in daily interaction with patients.

**Conclusions:** The ED is the vanguard of the hospital's claim to practice hospitality to all who are ill. That orientation itself has religious roots—compassion for neighbors who are sick or suffering—and ED clinicians' posture toward patients' spiritual concerns begins with this basic practice of attending carefully, rather than rationalization of the suffering. Practitioners can talk to patients about spiritual concerns whenever doing so is conducive to their primary goal of preserving and restoring the patient’s health—obviously not in the trauma bay in the midst of an emergency, but when time and clinical conditions allow.

The ED physician is not a spiritual therapist—it simply cannot be another responsibility or performance to chart in the health record, especially in the midst of escalating physician burnout rates—and there are chaplains and other professionals trained specifically for these interactions. ED clinicians, though, can attend to spiritual concerns because patients—and clinicians themselves—are spiritual beings and illness raises inescapable spiritual and existential questions. Offering a space for lament and questioning may be rewarding because doing so breaks the unfortunate default pattern of detached and instrumental interactions of contemporary healthcare. Additionally, spiritual engagement, irrespective of relevant intervention by the practitioner, may serve as a renewing of vocation for the clinician—the intrinsic value of serving the ill, which lies at the heart of medicine. The compassionate collaboration serves to restore patient flourishing while fostering clinician resilience by means of genuine personal connection. The goal of spiritual exploration is not technical competence, but rather practical wisdom through respectful candor, which can only be achieved through practice. While attention to spirituality should not get in the way of time-dependent and acute emergency medical care, an openness to and interest in spiritual concerns of patients may serve to honor those who are suffering. To invite the complexities of spirituality into the clinical encounter, even in the midst of many recognizable challenges of today's healthcare climate, ED practitioners must both reimagine and reframe what health means to themselves and the patients for whom they are caring.
Challenging Conversations: Medical Student Perspectives

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Background: Communication is the foundation of the doctor-patient relationship, thus communication skills training has been integrated into medical school curricula across the country. Training for difficult conversations is largely relegated to standardized patient encounters on end-of-life care and breaking bad news; rarely do curricula utilize other teaching methods or focus on additional aspects of challenging communication scenarios. Throughout their rotations, medical students frequently observe and participate in challenging conversations with patients; as of yet, it is unclear how students perceive these encounters. Understanding the medical student experience of challenging conversations with patients is necessary to enhance and diversify communication skills training. This study aims to characterize student perceptions of challenging conversations in neurology.

Methods: Medical students at Johns Hopkins School of Medicine completing a required four-week neurology clerkship were enrolled prospectively. At clerkship end, students were asked to reflect on the challenging conversations with patients they had experienced. In a survey, they were asked to identify the top three reasons those conversations were difficult. A subset of students participated in three semi-structured focus groups. Open-ended survey responses were qualitatively analyzed using conventional content analysis.

Results: Between August 2014 and March 2016, 159 medical students were enrolled (MS2: n=35, 22%; MS3: n=97, 61%; MS4: n=27, 17%). Three themes were identified: Clinical Reality: The content of conversations, such as bad news and poor prognosis, made encounters challenging. Students described how the contexts of conversations made them difficult, noting high stakes situations and time constraints. They discussed how clinical uncertainty contributes, often leaving providers unable to answer patients’ questions. Communication Skills Needs: Students found it hard to think of the right words to say, explaining the importance of tact and of avoiding jargon. They felt that students and providers lacked challenging conversation experience and training. Communicating the balance between honest and the provision of hope was difficult in these encounters. Navigating Emotions: Students discussed a variety of emotional reactions from patients, from the students themselves, and from providers. They felt unable to predict patients’ emotional reactions and noted the difficulty in choosing how best to respond to them. Students described feeling responsible for patients’ emotional and physical wellbeing, and the resultant feelings of inadequacy when they were not able to heal a patient.

Conclusions: Medical students identified modifiable and non-modifiable features that made conversations challenging. These findings elucidate how communication skills learning occurs via processes of experience-based learning in patient settings. Future curricula should educate on modifiable factors, such as honesty, hope, and emotions, and prepare students for non-modifiable aspects, such as uncertainty, breaking bad news, and the clinical environment. Curricula should include and emphasize real-time learning interventions to leverage the educational value of challenging conversations with patients, such as pre-conversation planning and post-conversation debriefing sessions.
Eviction as a Disruptive Factor in Health Care Utilization: Impact on Hospital Readmissions and No-show Rates

Peter Callejo-Black, Donna Biederman, Christian Douglas

Background: Residential eviction negatively affects health, but it is not known whether eviction disrupting health care utilization may be a mechanism.

Methods: We conducted a retrospective review of health care utilization of individuals who had been evicted from Durham public housing between January 2013 and December 2017. Hospital readmissions and no-show rates were investigated, looking at utilization within one year before and after eviction.

Results: There were 131 individuals who had been evicted and had one year of data pre- and post-eviction. The majority were African American (97.7%) and most were women (80.9%). There was a nonsignificant decrease in 30-, 60-, and 90-day hospital readmissions (p≥.05). No-show rate decreased from 27.57 per person per year to 20.13 (p≤.05).

Conclusions: For our study population, health care utilization was not disrupted significantly. The decreased no-show rate represents an opportunity for health systems to engage with patients on social factors affecting their health post-eviction.
Biofilm-dispersed Staphylococcus aureus Exhibits a Distinct agr-Independent Host Interaction

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Background: Staphylococcus aureus biofilms are a common cause of persistent, life-threatening infections. Dispersal of S. aureus cells from established biofilm-based infections is crucial for dissemination within the host, but is poorly understood. We tested the hypothesis that biofilm-dispersed S. aureus cells have distinct physiology from planktonic cells and are better equipped to evade host immunity in an agr-dependent manner.

Methods: Primary murine bone marrow derived macrophages (BMDMs) were infected with planktonic and biofilm-dispersed cells from S. aureus USA300 LAC wild-type (WT) and USA300 LAC-agr knockout (KO). Biofilm-dispersed cells were collected via glucose deprivation. Gentamicin protection assays were used to enumerate phagocytosed bacteria and fluorescence microscopy to quantify macrophage viability. A 26-plex immunoassay was used to screen for cytokines and chemokines. Reversed phase high-performance liquid chromatography was used to measure relative phenol-soluble modulin (PSM) levels from macrophage co-cultures.

Results: Compared with planktonic cells, biofilm-dispersed S. aureus in both WT and KO backgrounds exhibited ~10-fold less phagocytosis by BMDMs (p=0.0003), increased macrophage killing (23% vs. 8%; p=0.0038); stronger pro- (e.g. IFN-y, IL-6, IL-12p70, IL-17A, IL-22) and anti- (e.g. IL-4, IL-10, IL-13) inflammatory cytokine responses from macrophages (p<0.05 for all), weaker chemokine responses (e.g. IP-10, MCP-1, MIP-1α) primarily at 24 hours post-infection (p<0.05 for all), and significantly higher δ toxin PSM production (p=0.0090; Fig. 6) in WT background only.

Conclusions: S. aureus biofilm-dispersed cells are physiologically distinct from planktonic cells and have a unique host interaction that enhances survival against host immunity. Dispersed cells are more resistant to phagocytosis, have a greater propensity to kill macrophages, and mount stronger pro- and anti-inflammatory responses and weaker chemokines responses in an agr-independent manner. Dispersed cells also have the ability to produce more δ toxin PSM via well-known agr-dependent pathways. This study aids in guiding the treatment of biofilm-mediated infections and identifying therapeutic targets that optimize both the host immune system and antibiotic regimens. Further understanding the unique physiology of biofilm-dispersed S. aureus and the role of agr in host-pathogen interaction is essential for improving future patient outcomes.
Pilot Study Evaluating Innovative Educational Approach to Increase Knowledge of HIV Prevention in the Next Generation of Providers

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HVTN Research and Mentorship Program

Background: In the last several years, the overall rate of new HIV diagnoses in the US has declined, thanks to HIV testing, treatment as prevention (TasP), and advances in biomedical prevention like pre-exposure prophylaxis (PrEP) and post-exposure prophylaxis (PEP). Yet, men who have sex with men and transgender women remain disproportionately affected by HIV. Key to improving quality of prevention services are providers who are comfortable addressing concepts of HIV prevention and sexual health across the spectrums of gender identity and sexual orientation. We propose that for lasting impact, it is most important to begin HIV prevention and sexual health education before providers’ practice habits are established. We propose to address this need by creating interactive educational modules to increase HIV knowledge and awareness in future providers.

Methods: Columbia University Medical Center sexual health providers, research clinicians, community engagement professionals and New York City community members were consulted to develop a series of educational modules. Educational modules were piloted among medical students at Columbia University, Vagelos College of Physicians & Surgeons. Students in year 1 through 4 of medical school were eligible to participate. Knowledge of HIV and STI prevention and comfort assessing prevention needs of various patients were assessed via online 20-question questionnaire before and after educational intervention.

Results: One hundred twenty-three (123) students completed the pre-module questionnaire. Mean student age was 26.5 (25.0-28.0) and most identified as white, non-Hispanic (62/123; 50.4%) and heterosexual (96/123; 78.1%). Majority of students had heard of PrEP (122/123; 99.2%) and PEP (114/123; 92.7%) before the educational intervention. Eighty-nine (89) students completed the post-module questionnaire. Total mean score of LGBTQ familiarity increased from 3.6 (3.0-4.0) to 4.2 (4.0-4.7) – (p < 0.0001). Sexual history abilities score increased from 3.3 (3.0-3.7) to 3.6 (3.3-4.2) – (p = 0.002). Total mean score of HIV prevention confidence increased from 2.8 (2.6-3.0) to 4.2 (4.1-4.4) – (p < 0.0001). HIV and STI screening and prevention score increased from 6.4 (6.0-7.0) to 8.1 (7.0-9.0) – (p < 0.0001). Before educational intervention, 30.8% (38/123) of students agreed that they could confidently identify a patient who is a candidate for PrEP or PEP – this increased to 91% (81/89) following the educational intervention.

Conclusion: Our findings suggest that there is a need for increased HIV and STI prevention training in standard medical school curriculums that reflect all patients who exist along the spectrum of gender identity and sexual orientation and that educational modules are a feasible way of increasing medical student knowledge of HIV prevention.
Factors Associated with White and Non-White Patient Undergoing Peripheral Vascular Intervention for Peripheral Artery Disease.

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Background: Peripheral artery disease (PAD) is common and is associated with poor clinical outcomes, including major lower extremity amputation and all-cause death. While it affects all populations, worse outcomes have been observed in African-Americans. Over the last few decades, multiple novel technologies have been introduced to improve clinical outcomes in peripheral endovascular intervention (PVI). Drug coated balloons with Paclitaxel (DCB) were designed to reduce the occurrence of vessel restenosis, a common mechanism of revascularization failure in patients with PAD. Prior data has suggested there may be a lag in the diffusion and utility of cardiovascular therapies among underrepresented racial and ethnic populations due to things such as co-morbidities and socioeconomic status. The aim of this study is to explore patient factors that are associated with the use of DCB and clinical outcomes following PVI.

Methods: This is a retrospective analysis of patients undergoing femoropopliteal PVI, using Centers for Medicare and Medicaid Services inpatient, outpatient, and carrier administrative claims data from 2015-2016. All-cause mortality, all-cause hospitalization, repeat femoropopliteal revascularization and major lower extremity amputations at 1 year following PVI were the outcomes of interest. Patient factors associated with clinical outcomes were evaluated.

Results: A total of 83,225 patients underwent index femoropopliteal PVI with balloon angioplasty (DCB or standard) during the study period. A total of 67,049 (80.6%) patients were White while 16,176 (19.4%) were non-White. Non-White patients were younger, female and dually qualified for Medicaid when compared with white patients. Non-White patients had higher rates of chronic limb ischemia (45.2% vs. 36.0%), congestive heart failure (CHF) (44.7% vs. 36.5%), diabetes mellitus (75.9% vs. 58.3%) and renal disease (52.7% vs. 36.6%) when relative to White patients. Non-White patients with CHF (OR: 0.88 vs. 0.99, non-White 95% CI:0.81-0.96, White CI: 0.95-1.03) and myocardial infarction 0.82 vs. 0.95, non-White 95% CI: 0.75-0.91, White 95% CI: 0.91-0.99) had lower odds of receiving DCB compared with White patients. In addition, non-White patients in the inpatient setting had higher odds of receiving DCB compared to White patients. Non-White patients less frequently underwent PVI with DCB compared with White patients (26.7% vs. 29.9%).

Conclusions: Non-White patients in the study had higher rates of comorbidities compared with White patients. Interventions with DCB were associated with an overall improvement in outcomes, however, differential rates of use exist between non-White and White patients. Non-White patients had higher cumulative incidence and higher incidence with DCB of all-cause mortality, all-cause hospitalization, repeat femoropopliteal revascularization and major lower extremity amputation when compared with White patients. Regardless of standard interventions or DCB, non-White patients had worse outcomes compared with White patients.
Improving well-being amongst GME trainees: Building a partnership between GME and primary care can increase trainee access to primary care services

Christelle Tan, Kevin Shah, MD, Catherine Kuhn, MD, David Turner, MD

Background: Graduate medical education programs have sought to improve well-being of their trainees. While much emphasis has been appropriately placed on behavioral and mental health, GME trainees express difficulty maintaining routine primary care. The objective of this study is to report results of the implementation and utilization of a primary care concierge scheduling service and a primary care video visit service for GME trainees.

Methods: We evaluated implementation on three fronts. First, we quantified and characterized trainee demand for primary care services via review of recorded calls to the concierge scheduling line since 1 year post implementation. Second, we reviewed the volume, clinical characteristics and patient satisfaction for all completed video visits in the 1 year since implementation. Finally, we analyzed results of an annual survey sent to GME trainees to measure changes in well-being, primary care utilization, and delays in scheduling primary care appointments pre and post intervention.

Results: Out of 146 calls to the concierge line, 127 (87.0%) callers requested clinic appointments and 15 (10.3%) callers requested video appointments. Out of callers requesting clinic appointments 99 (80.0%) were scheduled. The leading reason for a clinic appointment not being scheduled was because the caller was asking to be seen by someone outside of primary care. 19 video visits were completed between October 2018 and April 2019. 100% of patient satisfaction survey respondents said they were satisfied with the video visit experience.

Conclusion: The concierge line was an effective way to connect trainees to primary care. Although the video visit platform worked well, low demand for video visits indicate that this is not the service trainees require. Concierge line for appointments outside of primary care, clinics closer to the hospital, clinic evening and weekend hours, and an asynchronous service are interventions that may better address healthcare needs.
The Patient Point-of-View: Characterizing patient-level factors associated with perceptions of health care

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Duke Center for Research to Advance Health (REACH) Equity (NIH: grant # U54MD012530) and the Eugene A. Stead Scholarship award

Background: The etiology of racial and ethnic disparities in healthcare is multifactorial, with patient, provider and system-level factors contributing to disparate outcomes. We explored patient-level values by characterizing the association between perceptions of care delivery and multiple factors that may influence patients’ assessment of care.

Methods: We surveyed participants from diverse racial backgrounds and education levels. We assessed patient values and perceptions of care using the Interpersonal Processes of Care (IPC) survey and validated instruments measuring five factors: trust, perceived empathy; stereotype threat; perceived everyday discrimination; and self-reported health. The IPC is a tool designed to assess quality of care based upon social-psychological components of the patient-physician interaction. It is divided into seven subdomains: (1) hurried communication, (2) elicited/responded to concerns, (3) explained results and medications, (4) patient-centered decision-making, (5) compassionate, respectful care, (6) discrimination, and (7) disrespectful office staff. We examined the associations between each subdomain of the IPC and the five patient-level factors via multiple linear regression. Analyses were adjusted for age, sex, race, ethnicity and education. We added a race interaction term to each model to assess whether associations between IPC subdomains and predictors differed by race (white vs. non-white).

Results: Of 50 participants, 80% were female with a mean age of 42.4 years (range 21-81). Participants were 40% Black, 52% White, and 22% identified as Hispanic/Latino ethnicity. In adjusted analyses, trust in the physician, perceived empathy from the provider and perceived everyday discrimination were significantly associated with most subdomains of the IPC. Specifically, greater perceived empathy in a clinical setting was associated with the perception that doctors have adequately elicited concerns, explained results and medications, engaged in patient-centered decision-making and demonstrated compassionate, respectful care (all p<0.01). Higher levels of trust in physicians were associated with perception that doctors have adequately elicited concerns (p=0.041), explained results and medications (p=0.0004), engaged in patient-centered decision-making (p=0.0001), and demonstrated compassionate, respectful care (p=0.0019). Greater perceived everyday discrimination was associated with perception of hurried communication (p=0.0108), disrespectful office staff (p=0.0062) and discriminatory behaviors (p=0.0261) in a clinical setting. The race interaction term was not significant in any of the models, indicating that race differences in these relationships were not apparent in our study population.

Conclusion: This exploratory study suggests that patient perception of quality care is significantly linked to empathy, trust and perceived everyday discrimination. Results present modifiable factors that may potentially improve patient perception and downstream health outcomes. Future research should focus on further characterizing factors associated with the IPC and determining the impact on health outcomes of interventions designed to improve processes of care.
Microbiology, Infectious Diseases and Immunology Study Program (MIDIP)
Antimicrobial and Sweat Gland-Associated Genes are Altered in Hidradenitis Suppurativa and Wounded Skin

Margaret Coates, Paula Mariottoni, David L. Corcoran, Hélène F. Kirshner, Tarannum Jaleel, David A. Brown, Stephen R. Brooks, John Murray, Maria I. Morasso, and Amanda S. MacLeod
Poindexter Scholarship in Basic Sciences

Background: Hidradenitis suppurativa (HS) is a debilitating chronic inflammatory skin disease resulting in non-healing wounds affecting body areas containing a high density of sweat glands and hair follicles. Cutaneous nodules form, which over time may rupture, resulting in dermal abscesses, sinus tracts, and open wounds. The pathogenesis of HS is not well understood but appears to involve aberrant activation of the innate immune system. Marked dysregulation of antimicrobial peptides and proteins (AMPs) is observed in HS, which may contribute to sustained inflammation. Despite the clinical similarities between HS lesions and cutaneous wounds, it is unknown whether there is overlap in the transcriptomes of these two conditions. We employed a biocomputational approach to examine the relationship between HS and innate antimicrobial defenses using a published data set (Blok et al., 2016). Additionally, we analyzed the gene expression signatures of a skin wound RNA-seq data set (Iglesias-Bartolome et al., 2018). Using differently expressed genes (DEGs) from both data sets, qPCR, and immunofluorescence, we showed that HS lesional skin and skin wounds share a number of upregulated DEGs. Conversely, we found that impaired gene and protein expression of sweat-gland associated genes is unique to HS, suggesting that eccrine sweat glands may contribute to HS pathology.

Methods: Microarray gene expression of HS lesional and non-lesional skin samples was re-analyzed (Blok et al., 2016). DEGs in HS lesional vs. non-lesional skin were also compared to DEGs in punch biopsy-wounded vs. normal skin (Iglesias-Bertolome et al., 2018). Using additional new patient-matched lesional and non-lesional skin samples we validated the findings via qPCR and protein immunofluorescence to confirm the transcriptional and protein expression of key DEGs.

Results: A number of AMPs, including members of the S100 family (log₂FC=4.85, p<0.001), defensins (log₂FC=3.75, p<0.001), and interferon stimulated antiviral genes, such as oligoadenylate synthetase 2 (log₂FC=1.88, p<0.001), were significantly upregulated in both HS lesional skin and wounded skin. In contrast, the AMP dermcidin (DCD) was among the most down-regulated genes in HS lesional skin (log₂FC=-4.90, p<0.001), but was up-regulated in wounded skin (log₂FC=5.26, p<0.001). Downregulation of DCD in HS lesions was confirmed via qPCR (log₂FC=-3.57, p<0.05) and immunofluorescence, which showed decreased expression of DCD in eccrine sweat glands of HS lesional skin, compared to HS non-lesional skin and normal skin from patients without HS. In addition, immunofluorescence revealed a markedly decreased number of eccrine sweat glands in HS lesional skin, compared to HS non-lesional and normal skin. Other sweat gland-associated genes, such as secretoglobins (log₂FC=-4.64, p<0.001) and aquaporin 5 (log₂FC=-1.69, p<0.01), had decreased expression in HS lesional skin and increased expression in wounded skin.

Conclusions: The increased expression of many AMPs in HS lesional skin and wounded skin suggests a similar inflammatory environment in both conditions. Conversely, our discovery that sweat-gland associated proteins, such as DCD, were decreased in HS lesional skin but not in wounded skin suggests that impaired sweat gland function may be a unique pathologic feature of HS.
Endemic typhoid incidence, Kilimanjaro Region, Tanzania, 2007-2018


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Background: Recent prospective surveillance studies have demonstrated a moderate to high incidence of typhoid fever in some settings in sub-Saharan Africa. Growing evidence suggests considerable variation in endemic typhoid incidence over time, yet few settings have multiple year incidence estimates to inform investments in typhoid control. We sought to describe a decade of variation in typhoid fever incidence in the Kilimanjaro Region of Tanzania.

Methods: A case of typhoid fever was defined as a blood culture positive for Salmonella enterica serovar Typhi in a febrile person. Cases were identified among febrile patients at two sentinel hospitals during three distinct periods: 2007-08, 2011-14, and 2016-18. To account for under-ascertainment at sentinel facilities, we derived adjustment multipliers from healthcare utilization surveys done in the catchment area of the sentinel hospitals in 2011 and 2018. Confidence intervals (CI) for incidence point estimates were derived using the 95% CI of our observed typhoid fever prevalence, of the healthcare seeking adjustment multiplier, and of published blood culture sensitivity.

Results: Of 3,556 participants, 50 typhoid fever cases were identified. Of typhoid cases, 26 (52%) were male and the median (range) age was 22 (<1-60) years; 4 (8%) were aged <5 years and 10 (20%) were aged 5 to 15 years. By time period, 32 (3.7%) of 870 cases enrolled in 2007-08, 15 (0.9%) of 1,753 in 2011-14, and 3 (0.3%) of 935 in 2016-18. Typhoid fever incidence was estimated as 60 (95% CI 15-283), 5 (95% CI 1-48), and 4 (95% CI 1-69) per 100,000 persons in 2007-08, 2011-14, and 2016-18, respectively. There were no deaths among cases.

Conclusions: We identified a moderate typhoid incidence point estimate in 2007-08 and low incidence point estimates during later surveillance periods, but with wide and overlapping CIs across study periods. Our data are consistent with evidence that endemic typhoid may vary substantially over time. Multiple year surveillance provides a clearer picture of typhoid incidence than single-year studies and may be warranted in locations making decisions about typhoid conjugate vaccine introduction and other control measures.
Neglected Infectious Diseases: confirming the role of tryptase as a clinical biomarker in Dengue & comparing Leptospirosis diagnostic tests

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Duke-Singapore Student Scholar Fellowship

Background: Dengue and Leptospirosis are examples of neglected tropical diseases (NTDs), a diverse group of infectious diseases that prevail in tropical regions and disproportionately affect rural and poor urban areas of low-income countries costing developing economies billions of dollars every year. To effectively lessen the burden of NTDs, we must explore all aspects of these diseases, including risk factors, epidemiology, diagnosis, pathogenesis, and treatment. Dengue virus (DENV) infection can cause Dengue Fever (DF), or the more severe Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS), characterized by hemorrhaging. Past research suggests that Dengue is immune-mediated. DENV activates mast cells (MC), which release several factors, including tryptase. Tryptase is thought to increase vascular leakage. We focused on confirming the role of tryptase as a biomarker of hemorrhage in DENV infection.

Leptospirosis is contracted by exposure to water contaminated by urine, blood, or tissue of animals infected with Leptospira bacteria, disproportionately affecting individuals depending on natural freshwater sources. In Sarawak, Malaysia, early diagnosis is challenging due to the lack of access to reliable diagnostic tests. We focused on comparing several current and new leptospirosis diagnostic tests.

Methods: To assess the correlation between hemorrhage in Dengue and tryptase levels, plasma tryptase levels of 40 dengue patients were measured using ELISA analysis. Samples were grouped based on the presence of hemorrhage at the time of study enrollment. To compare different leptospirosis diagnostic tests, blood and urine samples were obtained from patients presenting with leptospirosis symptoms at 3 hospitals in Sarawak, Malaysia. Leptorapide (RDT), MAT, real-time PCR, and ELISA analysis will be performed on these samples. A risk factor questionnaire and clinical presentation data were also collected to understand the epidemiology of leptospirosis in the region better.

Results: The average tryptase plasma concentrations of dengue patients with and without bleeding on initial presentation were 385.31 and 326.61 pg/mL (p=0.035). From the first 28 leptospirosis patients, 40% had a positive RDT, a significantly lower proportion were positive for MAT, and only 1 of the urine and none of the serum samples were positive on real-time PCR analysis.

Discussion: Tryptase showed a significant positive correlation with bleeding at initial presentation in Dengue patients. Further studies will be needed to assess its usefulness as a clinical biomarker in dengue patients and to understand its role in dengue pathogenesis and vascular leakage. The tests used for leptospirosis diagnosis showed a lack of consistency. The bacterial DNA amount in the samples was likely too low for our real-time PCR assay. Adapting the assay to detect lower concentrations and to better match local Leptospira strains should improve the results. Analysis of the risk factor and clinical presentation data should be performed to help with the prevention and diagnosis of Leptospirosis. Overall, these projects aim to tackle different needs of NTDs: the dengue project focuses on pathogenesis and treatment, whereas the leptospirosis project focuses on risk factors, epidemiology, and diagnosis. Collaborative efforts between clinical and basic scientists in different fields will be needed to obtain the most significant reduction in the burden of NTDs in Southeast Asia.
The impact of cytomegalovirus glycoprotein B (gB) antigenic variability on gB-specific non-neutralizing antibody binding and function

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Background: Human cytomegalovirus (HCMV) is the most common congenital infection, and the leading nongenetic cause of sensorineural hearing loss (SNHL) and neurodevelopmental delay in newborns globally. The most effective HCMV vaccine clinically tested to date, the subunit vaccine gB/MF59, demonstrated 50% efficacy in preventing infection in HCMV seronegative mothers. Our group has recently discovered that gB/MF59 vaccination elicited robust non-neutralizing antibody responses, that HCMV strains acquired by vaccinees more often included strains with gB genotypes that are distinct from the vaccine antigen than placebo recipients, and that protection against HCMV acquisition was correlated with ability of vaccine elicited antibodies to bind to cell membrane associated gB. Taken together, it is clear that future iterations of HCMV gB vaccines will need to consider gB antigenic variability and conformation. We hypothesize that gB specific non-neutralizing antibody binding and function are dependent on their epitope and genotype specificity as well as their ability to interact with membrane-associated gB.

Methods: 26 gB-specific monoclonal antibodies (mAbs) isolated from naturally HCMV infected individuals were screened for epitope specificity by binding antibody multiplex assay (BAMA), for genotype preference binding to cell associated gB via transfected cell binding, and for function by antibody dependent cellular phagocytosis (ADCP) and antibody dependent cellular cytotoxicity (ADCC). Moreover, to study conformational gB-specific antibody responses, sera obtained from rabbits vaccinated with full length soluble gB, gB ectodomain, or gB mRNA in lipid nanoparticles, were depleted for gB post fusion ectodomain specific antibodies. To determine the function of gB-specific Abs directed against prefusion gB, which would be expressed on the surface of a cell, versus postfusion soluble gB, postfusion gB-specific depleted samples were assessed for neutralization and ADCP function.

Results: This study identified gB-specific non-neutralizing mAbs primarily are specific for antigenic domain two (AD2) and Domain I. Further, variability in mAb gB genotype preference was observed with increased transfected cell binding to gB genotypes 2 and 4. Functional studies demonstrate two gB-specific monoclonal antibodies (mAbs) which facilitate antibody dependent cellular phagocytosis (ADCP) in THP1 monocyte like cells and primary monocytes isolated from healthy donors. These ADCP mediating mAbs have binding specificities of AD2 and Domain I respectively. Interestingly, ADCP, but not neutralization, was retained in postfusion gB depleted plasma of vaccinees who received either soluble full length gB or gB mRNA LN, but not gB ectodomain.

Conclusions: This investigation provides novel understanding on the impact of gB antigenic variability on critical gB-specific non-neutralizing antibody responses, ultimately guiding the design of next generation CMV vaccines.
Inhibition of Myeloid Cell Trafficking Enhances Natural Killer Cell Immunotherapy

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NIH Medical Research Scholars Program Fellowship

Background: Immunotherapies that activate or replace T-cell effector immunity are limited by the ability of subpopulations of tumor cells to escape T-cell immunity through genomic alterations that abrogate T cell antigen processing and presentation. Natural killer (NK) cell-based immunotherapy may overcome these limitations through antigen- and MHC class I-independent tumor cell recognition and killing, particularly in tumors that display a non-T-cell inflamed phenotype. Cellular immunity is also limited by immunosuppression within the tumor microenvironment, mediated in part by myeloid derived suppressor cells (MDSCs). MDSCs clearly suppress the function adoptively transferred T cells, but whether MDSCs also abrogate adoptively transferred NK cell function is poorly understood. Here, we hypothesized that MDSCs limit the effector function of NK cells and that abrogation of MDSC tumor trafficking with SX-682, a chemokine small molecule inhibitor, would enhance the efficacy of NK cellular therapy.

Methods: Mouse oral carcinoma 2 (MOC2) is a syngeneic model of head and neck squamous cell carcinoma (HNSCC) that forms non-T-cell inflamed tumors in wild-type B6 mice. All animal work was approved by the NIH Animal Care and Use Committee. KIL, a culturable NK cell line derived from B6 mice, was used as a source of NK cells. SX-682 is an orally bioavailable small molecule dual inhibitor of CXCR1/2. Flow cytometric analysis, magnetic sorting, real-time impedance analysis and ELISpot assays were used to assess NK cells and MDSCs from murine and human tissues.

Results: Mice bearing non-T-cell inflamed MOC2 tumors significantly accumulated peripheral CXCR2+ Ly6G+ neutrophilic myeloid cells compared to non-tumor bearing mice. Trafficking of Ly6G+ myeloid cells led to significant MOC2 tumor infiltration by day 14. Sorted peripheral and tumor infiltrating Ly6G+ myeloid cells suppressed KIL effector function in a dose-dependent fashion, validating them as neutrophilic-MDSC (PMN-MDSC). Tumor PMN-MDSC suppression of KIL cells was mediated by TGF- and reactive oxygen species. Treatment of mice with SX-682 significantly abrogated tumor trafficking of PMN-MDSCs and enhanced tumor infiltration and the function of adoptively transferred KIL. Although SX-682 treatment and adoptive transfer of KIL alone produced no tumor growth inhibition (TGI), combination treatment resulted in significant TGI and prolonged survival of mice bearing MOC2 tumors. SX-682 had no direct effect on MOC2 tumor cell proliferation, viability, motility or susceptibility to NK cell lysis, suggesting that the ability of SX-682 to sensitize MOC2 tumors to NK cellular therapy was primarily due to inhibition of PMN-MDSC tumor trafficking. Tumors from patients with HNSCC accumulated CXCR1/2+ CD15+ and CD14+ myeloid cells. Sorted CD15+ and CD14+ myeloid cells suppressed the activation of healthy donor NK cells through TGF- and nitric oxide synthase function, respectively, validating the infiltration of MDSC that suppress NK cell function into human HNSCC tumors.

Conclusions: Murine MDSC inhibited the effector function of NK cells, and abrogation of MDSC tumor trafficking enhanced the efficacy of KIL adoptive transfer treatment. Peripheral and tumor infiltrating MDSC from HNSCC patients suppressed the function of healthy donor NK. Multiple, non-overlapping mechanisms of MDSC suppression suggest that inhibition of MDSC trafficking into tumors with chemokine inhibition may be the most effective approach to reversing MDSC-mediated local immunosuppression. These data provide the rationale for the clinical investigation of myeloid chemokine inhibitors in combination with NK cell-based immunotherapy, particularly in patients harboring non-T-cell inflamed tumors.
Murine Models for the Host Response to Typical and Atypical Pneumonia

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Background: The etiology of pneumonia is difficult to diagnose, with typical bacterial, atypical bacterial, and viral infections being the most common causes. However, diagnostics that discriminate these infectious etiologies are limited. We, therefore, focused on the host response to identify possible diagnostic markers and better understand these infections. However, atypical bacterial pneumonia is challenging to identify in humans precisely because of this diagnostic difficulty. Therefore, we utilized murine models to define host response differences between typical bacterial, atypical bacterial, and viral pneumonia.

Methods: Mice were intranasally inoculated with S. pneumoniae (n=38), M. pneumoniae (n=27), H1N1 pr8 (n=19), or saline as a control (n=42). RNA was extracted from peripheral blood collected at 24 h, 48 h, 72 h, 120 h, or 168 h and subjected to microarray analysis. Diagnostic signatures were generated using lasso logistic regression and accuracy was assessed using nested leave-one-out cross-validation with feature selection repeated within each iteration. Differentially expressed genes were used to perform gene set enrichment analysis. These murine-derived signatures were externally validated in silico in 487 human subjects found across 5 publicly available data sets.

Results: We generated pathogen-specific murine disease signatures that performed with 91-100% accuracy. Pathway analysis revealed that animals with pneumococcal pneumonia had a robust immune response by 48 hours that continued to 72-hours post-infection. In contrast, animals infected with M. pneumoniae did not show evidence of a strong immune response until 72-hours post-infection. Additionally, the immune response to M. pneumoniae bared greater similarity to the viral response than it did to the host pneumococcal response. H1N1- infected mice showed an anti-viral response at 120 hours that resolved by 168 hours post-infection. The AUC values resulting from independent human validation of our murine signatures ranged from 89-98%.

Conclusions: There are discrete host responses to typical bacterial, atypical bacterial, and viral etiologies of pneumonia in mice. These signatures validate well in humans, highlighting the conserved nature of the host response to these pathogen classes.
Development of Broadly Neutralizing Antibodies in HIV-1 Infected Children

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Doris Duke Clinical Research Mentorship Scholarship

Background: Adolescents represent the only age group that has experienced an increase in AIDS-related deaths over the last decade. In 2017, 590,000 young adults between the ages of 15-24 became newly infected with HIV. This underscores the importance for an HIV vaccine tailored to the pediatric population and delivered prior to sexual debut. Elicitation of antibodies capable of neutralizing multiple HIV-1 variants (broadly neutralizing antibodies (bnAbs)) is believed to be a critical component of a protective vaccine. While some chronically infected adults develop bnAbs several years after infection, no vaccine developed to date has been able to elicit this response. Recent studies conducted in small cohorts have indicated that bnAb responses may develop earlier in children. Confirming and investigating this response in a larger cohort will provide important insights on bnAb development in children, informing our development of a pediatric HIV vaccine.

Methods: The magnitude, epitope specificity and IgG subclass distribution of HIV Envelope (Env)-specific antibodies were assessed in 212 ART-naïve HIV clade B infected children aged 1 to 3 years using a binding antibody multiplex assay (BAMA). Samples from 44 clade B chronically infected adults were tested with the same BAMA panel to compare antibody profiles between adults and children. In addition, the neutralizing activity of the pediatric samples was assessed against a panel of 10 tier-2 viruses from multiple clades. Neutralization breadth and potency were compared to those of chronically infected adults using a published dataset. Broadly neutralizing pediatric samples were then assayed against mutant viruses to identify key epitopes responsible for neutralization.

Results: The levels of HIV Env-specific binding antibodies increased from age 1 to 2, and levels in 2 and 3-year-old children were comparable to those of adults. Overall, there was no significant difference in the specificities of binding antibodies between adults and children. Similarly, there were no significant differences in the IgG subclass distribution between pediatric and adult cohorts. The 1-year-old cohort demonstrated neutralization breadth that was comparable to that of chronically infected adults, and this breadth continued to increase with age such that the pediatric cohort overall exhibited significantly greater neutralization breadth than chronically infected adults. In five of the pediatric samples, neutralization activity was mapped to a single epitope (CD4 binding site, V2 or V3 glycans). However, epitope specificity of neutralizing antibodies could not be mapped for the majority of samples, suggesting that neutralization may be mediated by a polyclonal response.

Conclusions: Our results indicate that children are able to mount an Env-specific antibody binding response to HIV infection that is as robust as what is observed in adults and also confirm previous works indicating that children develop cross-clade neutralizing antibodies earlier than adults. Contrary to adults, broad neutralization in children may be mediated by a polyclonal response, suggesting differences in pathways of induction of neutralization breadth in adults and children. In future work, we will isolate and characterize HIV-specific monoclonal antibodies from infected children and compare their immunogenetic characteristics to those of adults.
Comparison of Multiple Host Response-Based Strategies to Classify Acute Respiratory Illness

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BACKGROUND: Host response-based diagnostics are an alternative to pathogen based tests. Host response strategies include proteomic and transcriptomic approaches. Here, we compare three host response strategies for ARI diagnosis: Procalcitonin (PCT), a 3-protein panel, and an mRNA panel.

METHODS: PCT, a 3-protein panel (CRP, IP-10, TRAIL), and a host gene expression mRNA panel were measured in a cohort of 286 participants presenting to one of four Emergency Departments with ARI due to bacterial (n=47), viral (n=162), or non-infectious (n=77) etiologies. Multinomial logistic regression and leave-one-out cross validation were used to train and evaluate the protein and mRNA panels. Performance characteristics were calculated for each method, and their combination, for the ability to discriminate bacterial vs non-bacterial infection and viral vs non-viral infection. PCT was not evaluated for viral vs. non-viral discrimination since it does not discriminate viral and non-infectious etiologies. McNemar’s test was used to compare overall accuracy of mRNA and protein panels.

RESULTS: For discriminating bacterial vs non-bacterial etiologies, the mRNA panel had an AUC of 0.93 vs 0.83 for both the protein panel and PCT. A model utilizing all three strategies was the same as mRNA alone. Using previously established cutoffs, overall accuracy was similar between mRNA and protein panels, but the protein panel had widely discordant sensitivity (43%) and specificity (92%). When selecting an optimal cutoff for the protein panel that balanced the two (82% and 73%, respectively), the mRNA panel had a significantly greater overall accuracy (p < 0.001). Similar results were found when discriminating viral vs. non-viral subjects: the mRNA panel (AUC = 0.93) outperformed the protein panel (AUC = 0.84). Combining the mRNA and protein panels was equivalent to the mRNA panel alone.

CONCLUSIONS: A host-based gene expression signature is the most effective platform for classifying subjects with bacterial, viral, or non-infectious ARI. A gene expression approach, when translated to a clinically available platform, may facilitate diagnosis and clinical management of acute infectious diseases, mitigating antibiotic overuse.

Figure 1: Bacterial (a) and viral (b) probability receiver operating characteristic curves for different host biomarker strategies either alone or in combination.
Market Risk Factors for Detection of Avian Influenza in Guangdong, China

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Background: Guangdong province in China has been a hotbed of emerging zoonotic disease, and the large, mixed-species poultry markets frequented by significant portions of the population have provided a fertile ground for the spread and development of avian influenza. Emergence of zoonotic H5N1 and HPAI H7N9 have both been traced to Guangdong, and the province’s extreme density of people and poultry make it a high probability area for spillover. However, there are multiple types of markets in the live bird market (LBM) ecosystem—from small retail markets to larger wholesale markets—and the risk factors that contribute to the presence of AIVs within these markets are still being elucidated. Understanding of these risk factors can help local health workers target the areas of greatest exposure hazard and reduce the chance for animal to animal transmission and human spillover.

Methods: We collected a large pool of environmental and animal samples (n=14,358) over a diverse geographic region of Guangdong province over the course of 18-23 months. Throat, cloaca and environmental samples were collected from 18 different markets over the course of 23 months. Samples were tested for Influenza A using RT-PCR, followed by further subtyping for A(H5), A(H7) and A(H9). Data was then analyzed using univariate and multivariable logistic analysis.

Results: We demonstrated that markets in Guangdong continue to be contaminated with circulating A(H5), A(H7) and A(H9) serotypes with a combined prevalence of 1.7%, 0.5%, 10.5% respectively. There was a drop in A(H7) prevalence from 1.02% to 0.3% following initiation of vaccination in August 2017. Multivariable logistic regression analysis showed that there was an increased odds of positive sampling for all tested subtypes in winter months, and an increase of A(H7) and untyped influenza in the spring period. Additionally, A(H5) and A(H7) were significantly more prevalent in wholesale markets than retail markets. There was also species variability, with waterfowl such as ducks and goose having significantly higher odds of being infected with A(H5) and un-identified non-A(H5)/A(H7)/A(H9) subtypes, but significantly lower rates of A(H9). Co-infection among waterfowl was also higher, and there was significantly more A(H7) and AH(9) co-infection than any other subtype and nearly as many A(H7)/A(H9) co-infections as single order A(H7) infection. Analysis of environmental sampling showed items involved in butchering have the highest risk of contamination with AIV, and that there may be less A(H9) detectable by RT-PCR in the environment than other subtypes, but that this pattern does not necessarily extend to oropharyngeal swabs.

Conclusions: Our analysis suggests that there are appreciable differences in the spectrum of subtypes that market species harbor, that there are areas within markets that pose a greater risk for exposure to subtypes of concern and that there is co-circulation which may increase the chances for gene reassortment between subtypes. There also appears to be consistent, low-level, contamination with A(H5), A(H7) and A(H9) despite vaccination. Our data makes a case for continued careful surveillance of waterfowl and their role in emerging AIV strains, a greater attention to the strict geographic separation of species within large markets, and modifications to the way poultry meat is butchered in markets, including discouraging wooden tools and encouraging cleaning multiple times a day.
Cardiovascular Study Program (CVS)
Human Genetics and Genomics Study Program (HGP)
Molecular Medicine Study Program (MolMed)
Medical Scientist Training Program (MSTP)
Pathology Study Program (PSP)
Radiology, Radiation Oncology, and Medical Physics (RROMP)
Investigating the Role of Dendritic Cell IL-1 Receptor in T cell Effector Function

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Eugene A. Stead Student Research Scholarship

Background: Acute kidney injury (AKI) is a frequent in-hospital complication that significantly increases morbidity and mortality, with an overall hospital morality rate of 62%. Unresolved, AKI promotes renal fibrosis and chronic kidney disease (CKD), a major risk factor for future cardiovascular events. One novel therapeutic approach to promote post-AKI healing is to modulate the functions of immune cells involved in kidney injury. Dendritic cells (DCs) are antigen presenting cells (APCs) that release cytokines upon activation to enhance the innate immune response and activate naive T lymphocytes to promote the initiation of adaptive immunity. Several studies have demonstrated either a protective or deleterious effect of DCs in kidney injury, varying with the nature of the injury. One possibility for this difference is that pro-inflammatory cytokines, such as IL-1, modulate the microenvironment to influence DC–T cell interactions to modulate kidney injury and healing. Previous studies have demonstrated that IL-1 receptor (IL-1R1) KO mice have reduced inflammation, less kidney injury, and increased rates of recovery following ischemic AKI. Therefore, we hypothesized that the IL1 receptor on dendritic cells is involved in DC maturation and subsequently leads to activation of inflammatory effector T cells.

Methods: To investigate the role of IL1R1 present on DCs and potential effects on T cell effector function, bone marrow from global IL1R1 KO mice was harvested and cultured via GM-CSF to establish bone marrow derived DCs. We then analyzed these DCs for maturation makers (CD40, CD86, CCR7, MHCII). To analyze effects of DC IL-1R1 on T cell function, DCs were pulsed with ovalbumin peptide and stimulated with IL-1 and co-cultured with CD4+ T cells from OTII mice specific for ovalbumin peptide. We then analyzed effects on T cell proliferation, apoptosis and survival, and T cell polarization to various T helper subsets (Th1, Th2, FoxP3, Treg).

Results: We found that IL1 induces DC maturation via IL1R1, which appears to alter T cell function. KO DCs had reduced expression of CD40 (p<0.0001), CD86 (p=0.05), CCR7 (p=0.0041), and MHCII (p=0.04). Furthermore, KO DCs appear to be less effective at supporting CD4+ T cell proliferation. After 3 days of coculture, the number of T cells was greatly reduced when cultured with KO DCs pulsed with peptide and stimulated with IL-1 (p = 0.0014); and at 5 days, there was a trend in the ratio of the number of T cells that had undergone three or more divisions to the number of undivided T cells in the WT group compared to the KO group. When compared to KO DCs, WT DCs also induced a strong trend towards increased numbers of proliferated IFN OT-2 CD4+ T cells, indicating a potential tendency towards an inflammatory Th1 phenotype.

Conclusions: These studies provide evidence that IL1 is able to induce maturation of DCs through the IL1R1, which appears to promote T cell proliferation and the ability to activate and polarize to Th1 effector function. Our work provides an important foundation to understanding the mechanisms that underlie the effects of DC-T interactions in influencing kidney injury, which could inform future therapies to alleviate the morbidity associated with AKI.
Video review for reflective practice of high-fidelity simulation performance in undergraduate medical education

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**Background:** While engaging in reflective practice is an important component, it has been extensively shown that healthcare students and providers demonstrate poor correlation between performance and self-assessment. When combined with feedback from peer groups or from clinical faculty, self-assessment aligns more closely with externally graded performance. However, the time and resources required to provide this type of individual guided feedback limit its practical utility in undergraduate medical education. In this study, we sought to determine the self-assessment profile of higher versus lower performing students in an Objective Structured Clinical Examination. In addition, we sought to determine the role of individual video review in enhancing self-assessment.

**Methods:** This was a retrospective study of self-assessment data collected from all students who took the Duke Clinical Performance Exam (CPX) between 2014 and 2018 (N = 554). Students completed an initial survey immediately following each of two selected testing stations, and the same survey again after watching a video recording of their performance on the station. Categorical survey data were assigned numerical values, and a self-assessment score was calculated based on total possible survey points. Students were ranked based on their graded CPX scores using both total CPX score and overall interpersonal skills score. Higher performing students were defined as those with scores ≥1 standard deviation above the mean and lower performing students as those with scores ≥1 standard deviation below the mean. Statistical analysis was performed for the 361 students who completed a pre-video and post-video self-assessment for at least one station. Differences between higher performers and lower performers were calculated using one-way ANOVA. Differences between pre- and post-video review self-assessment scores within each group were calculated using a paired t-test.

**Results:** There was no significant difference in the absolute self-assessment scores of higher versus lower performers on the Clinical Performance Exam either before or after video review, nor was there any correlation between self-assessment scores and exam scores. Higher performing students did demonstrate a significant difference in their self-assessment scores before and after video review. This difference was found both in the diagnostic and the counseling scenarios, regardless of whether students were ranked based on total score or interpersonal score. Conversely, there was no difference between the pre- and post-video review self-assessment scores of lower performing students.

**Conclusions:** Higher performing students have a significant decrease in self-assessment scores after video review, while lower-performing students have no significant change. This difference was seen in the absence of guided video evaluation or external feedback on individual student performance. Higher performing students are better able to evaluate and reflect on their performance using video review. While all students would likely benefit from guided feedback with faculty, in the setting of limited resources, these should be directed toward lower performing students, for whom independent video review has less impact on the ability to self-assess.
Regulation of the Mesothelial-to-Mesenchymal Transition in Omental Mesothelial Cells by the Ovarian Cancer Secretome

Helen Daifotis BA, Abir Mukherjee PhD, Ernst Lengyel MD PhD

Background: In the case of ovarian cancer (OvCa), those diagnosed with late-stage disease have an estimated 5-year survival of less than 40%. This is important because close to 80% of women with high-grade serous ovarian cancer, the most common subtype of OvCa, have late-stage disease at the time of diagnosis. Here, we aim to characterize the signaling pathways that drive the interaction between metastasizing OvCa cells and the omentum, a 20x13x3 cm fat pad which serves as the primary metastatic site.

Methods: To determine whether the OvCa secretome is capable of inducing a mesothelial- to-mesenchymal transition (MMT) in omental mesothelial cells, representative cell lines (Tyknu, HeyA8, Ovcar5, and Kuramochi) were grown as spheroids and the conditioned media was isolated. HPMCs were then treated with the media and mesenchymal changes were assessed via fluorescent staining and analysis of protein expression. To determine how much of this transition may be attributed to the TGF-β ligands themselves, we assessed for whether or not each isoform was individually capable of inducing MMT. We then systematically sequestered each isoform of TGF-β following conditioned media treatment to establish the role that each isoform might play in OvCa. To determine how much of each isoform was present in the conditioned media and whether the abundance of any isoform correlated with MMT induction, we performed an enzyme-linked immunosorbent assay (ELISA) to assess for the levels of each isoform in both conditioned media and in ascites from patients with HGSOC. Finally, we performed neutral lipid staining and cholesterol treatment to investigate an alternative TGF-β-independent pathway that may be important for MMT signaling.

Results: We demonstrated that the OvCa secretome is capable of inducing MMT in human primary mesothelial cells (HPMCs). Treatment of HPMCs with cancer conditioned media led to a decrease in epithelial markers E-cadherin) and an increase in mesenchymal markers (snail). This transformation was accompanied by increased stress fiber formation and loss of the cobblestone appearance associated with normal HPMCs. ELISA data supported at least partial independence from TGF-β in this transformation. While TGF-β 1 was the predominant isoform present in ascites, Tyknu conditioned media, a serous cell line, that was lower in TGF-β 1 was still capable of inducing MMT versus HeyA8 conditioned media, a non-serous cell line, that contained a greater amount of TGF-β 1. TGF-β 3 was not detectable in either ascites or conditioned media and TGF-β 2 was present in low abundance. Sequestration of TGF-β 2 or TGF-β 3 did not prevent MMT induction Metabolomics previously conducted in the lab demonstrated a unique metabolic signature associated with MMT characterized by alterations in fatty acids and cholesterol esters. Preliminary data presented here suggests a correlation between intracellular neutral lipid droplets and MMT.

Conclusions: Our results demonstrate that TGF-β 1, TGF-β 2, and TGF-β 3 levels do not directly correlate with MMT of omental mesothelial cells in the setting of disseminated OvCa. We show that while TGF- β isoforms are capable of inducing MMT, there is likely an additional TGF- β-independent component involved in this transformation. Furthermore, we provide evidence that TGF- β-independent signaling leads to changes in neutral lipids, such as cholesterol esters and fatty acids, that are correlated with MMT induction in omental mesothelial cells.
HIF-2α-Mediated Vascular Ocular Anomalies in EPAS1 Gain-of-Function Syndrome

Pauline Dmitriev, BS, Herui Wang, PhD, Emily Chew, MD, Karel Pacak, MD, PhD, Zhengping Zhuang, MD, PhD
NIH Intramural Research Program

Background: Normal development of the mammalian embryo occurs in the context of hypoxia. Consequently, hypoxia-inducible transcription factors (HIFs) are critically important in development. We recently described a new syndrome (EPAS1 gain-of-function mutation syndrome; Pacak-Zhuang syndrome) that is caused by somatic mosaicism of a gain-of-function mutation of EPAS1 which encodes hypoxia-inducible factor 2 (HIF-2α). Affected patients present with multiple paragangliomas/pheochromocytomas, duodenal somatostatinoma, polycythemia, headaches, and diminished visual acuity at an early age. The characteristic phenotype and established genetic cause of the syndrome provides a unique opportunity to study the role HIF-2α plays in oxygen sensing, development in regions of physiologic hypoxia, and other pathological processes. In this study, we describe novel ocular lesions in patients with EPAS1-gain-of-function mutation syndrome and aim to determine if early-onset diminished visual acuity is developmental in nature or related to long-term physiologic sequelae of the syndrome.

Methods: Patients were referred to the NIH for evaluation of recurrent and metastatic paragangliomas or pheochromocytomas, accompanied by polycythemia. Patients were confirmed by identification of the EPAS1 gain-of-function mutation in resected tumors and other tissues. Epas1A529V mutant mice (corresponding to human EPAS1A530V) were established by TALEN-mediated homologous recombination. Both patients and mice underwent ophthalmic evaluation with fundoscopy, fluorescein angiography (FA), and enhanced-depth imaging optical coherence tomography (EDI-OCT). Whole mount immunofluorescent staining of neonatal mouse retinas with isolectin B4 was used to further investigate the role of HIF-2α in angiogenesis and vascular patterning in vivo.

Results: All patients were found to have ocular abnormalities. Fundoscopy demonstrated fibrosis overlying the optic disc, tortuous and dilated retinal vessels, and retinal pigment epithelium changes. Optic disc edema and retinal exudates were also seen. Fluorescein angiography showed leakage of dye from post-capillary venules surrounding the optic disc and highlighted the aberrant retinal vascular patterns. Enhanced-depth imaging optical coherence tomography demonstrated significant thickening of the choroid and dilation of choroidal vessels. Ophthalmic evaluation of mice in our transgenic gain-of-function Epas1A529V mutant mouse model recapitulated the above findings. Furthermore, immunofluorescent staining of neonatal mouse retinas with isolectin B4 highlighted the pathologic angiogenesis and abnormal development of retinal vasculature occurring in mutant retinas as compared to littermate controls.

Conclusions: Our findings demonstrate the important role of HIF-2α and hypoxia signaling in vessel development within the choroid and retina. We conclude that marked permanent choroidal thickening, without traumatic pachychoroidopathy, and tortuous and dilated veins seen in the choroid and retina in patients with EPAS1 gain-of-function mutation syndrome are indicative of persistence of venous elements within developing mesenchyme. These results underscore the crucial role hypoxia signaling plays in the development of hypoxia-dependent tissues and may help explain other eye and vascular abnormalities whose pathogenesis is presently unknown.
Identifying novel mechanisms of p53-mediated tumor suppression

Nathan H. Leisenring, Robert W. Floyd, David G. Kirsch

**Background:** TP53 is the most frequently mutated gene in human cancer. Classically, p53-mediated tumor suppression is thought to occur via transactivation of canonical targets that induce cell cycle arrest and apoptosis. However, this model has been challenged by studies using mouse p53 mutants. The mouse transactivation domain mutant, p53<sup>25,26</sup>, is unable to activate most canonical p53 targets and fails to induce cell cycle arrest or apoptosis in response to DNA damage. However, it retains the ability to suppress tumor development in vivo in mouse models. Similarly, some human p53 mutants are found in Li-Fraumeni syndrome and in sequenced tumors despite retaining the ability to transactivate canonical targets, while other human p53 mutants are not found in cancer despite loss of transactivation. The goal of our study is to identify novel p53 functions essential for tumor suppression by evaluating human p53 mutations where transactivation of classical targets is uncoupled from tumor suppression activity.

**Methods:** To identify human TP53 missense mutants with tumor suppressive function dissociated from the ability to activate canonical p53 pathways, we first utilized the results of a 2003 saturation mutagenesis screen performed in yeast which stratified human p53 mutants by their ability to activate 8 canonical p53 targets. We then cross-referenced these results with tumor sequencing data from the IARC, the TCGA PanCancer Atlas, and AACR GENIE. We identified panels of human p53 missense mutants which either 1) retained transactivation ability in yeast, yet had been identified in sequenced human tumors or 2) were unable to transactivate p53 targets in yeast, but had not been identified in sequenced humantumors.

To study these p53 mutants compared to wt p53, we cloned their sequences into doxycycline-inducible pLVX-TetOne-Puro plasmids and puromycin-selected for cell lines stably expressing the construct in p53 null H1299 and HCT116 cells. Using qPCR and western blots we validated the expression of mutant or wt p53 in these human cell lines and assessed for transactivation of canonical targets p21, mdm2, puma, gadd45, and 14-3-3. The IncuCyte Live Cell Analysis system and XTT Cell Proliferation assays were used to evaluate p53-mediated growth arrest of human cancer cells following induction of each mutant.

**Results:** From these experiments, we selected two mutants for further study. Missense p53 mutant E224D is found in human cancer and in the germline of Li-Fraumeni syndrome patients yet retains the capacity to transactivate classical p53 targets and arrest tumor cell growth in vitro. In contrast, p53 mutant G262A is not found in cancer but lacks the ability to activate p53 targets and fails to arrest tumor cell growth in vitro.

**Conclusions:** To dissect the in vivo tumor suppressive function of these mutants, we are developing transgenic mice expressing our two p53 mutants of interest. The results of this ongoing study may uncover novel mechanisms of p53-mediated tumor suppression.
**Gastrin Signaling in Esophageal Submucosal Glands**


*NIH supplementary T-32 training grant (T32-28S2)*

**Background:** Barrett’s Esophagus (BE) is characterized by esophageal metaplasia from stratified squamous epithelium to an intestinal phenotype. BE is a predisposing factor to esophageal adenocarcinoma (EAC), which has a 5-year survival rate of 19.7% and increasing incidence. BE likely arises as a response of abnormal healing, yet the cellular origin of BE is still debated. One theory is that BE may arise from progenitors within esophageal submucosal glands (ESMGs). When squamous epithelium is injured (as in the case of acid reflux), ESMGs demonstrate the proliferative, ductal phenotype known as acinar ductal metaplasia (ADM). We have previously shown an association of ADM with esophageal injury, BE and EAC; ADM is a cancer precursor in other organs. The factors that lead to ADM in ESMGs are unknown. Proton pump inhibitors (PPIs) are recommended for BE by current guidelines, yet PPI use increases serum gastrin. Interestingly, hypergastrinemia and gastrin receptor (CCKBR) up-regulation have recently been associated with increased proliferation in BE. We hypothesized that elevated gastrin signaling in ESMGs may contribute to development of esophageal ADM.

**Methods:** To investigate the correlation between ESMG ADM and CCKBR expression, we used a human esophagectomy database containing patients with varying types of esophageal epithelium (squamous, inflammation/ulcer, BE, EAC) and performed immunohistochemistry (IHC) for CCKBR. Similarly, we used an *in vivo* porcine model with injury via radiofrequency ablation to compare CCKBR expression in normal vs. injury. We also treated 3D ESMG spheroids with gastrin and then determined changes in ESMG proliferation and phenotype *in vitro*.

**Results:** At baseline, very little CCKBR was identified in ESMGs. However, in human ESMGs, CCKBR expression increased in association with BE and EAC. When CCKBR was present in human ESMGs, it was primarily in acini with a ductal phenotype, consistent with ADM. When gastrin was added to 3D ESMG culture, total mean hollow spheroid count and the mean percent hollow phenotype increased. When gastrin inhibitor was added to gastrin treated spheroids the effect was reduced.

**Conclusions:** These novel data implicate gastrin/CCKBR signaling response to injury in ESMGs. While it is known that CCKBR is absent in uninjured squamous epithelium but present in BE and EAC, we have added to this knowledge by demonstrating CCKBR in activated ESMGs as part of the proliferative progenitor phenotype seen in ADM.
A High-Throughput Screen Identifies Potential Chemotherapeutics for EGFRvIII+ Glioblastoma

Sarah Nullmeyer, Cory Nanni, Madan Kwatra, PhD

**Background:** Glioblastoma (GBM) is the most common and deadly brain tumor in adults, accounting for over 45% of all primary malignant brain cancers. The defining histological features of necrosis and microvascular proliferation qualify GBM for the WHO's highest histological grade IV classification. The standard of care currently consists of maximal surgical resection, followed by postoperative radiation and concurrent therapy with the alkylating chemotherapeutic agent temozolomide. Despite this regimen, median survival is still only 14.6 months after initial diagnosis and fewer than 5% of patients survive more than 5 years. Further, survival rates have not appreciably increased in the last 30 years. Clearly new therapies are needed if outcomes are to improve. Towards this goal, the aim of this study was to identify anti-cancer agents effective against glioblastomas that express the EGFRvIII mutation, a cancer-specific mutation expressed in over 20% of GBMs.

**Methods:** We screened two EGFRvIII-expressing glioblastoma stem cell lines against a panel of 119 anti-cancer agents and identified two chemotherapeutics, gemcitabine and cladribine, as potential agents. Both agents were tested in *in vitro* cell viability, invasion, and cell death assays using multiple EGFRvIII-expressing GBM stem cell lines. Gemcitabine and cladribine were also tested in *in vivo* tumor growth inhibition and survival experiments using mice models. Twenty athymic mice were used for *in vivo* experiments, with five mice in each of the following treatment groups: cladribine control, cladribine experimental, gemcitabine control, and gemcitabine experimental. Statistical significance (p< 0.05) was evaluated using one-way ANOVA followed by Tukey's Honest Significant Difference Test for cell death and invasion assays, a Student’s Unpaired T-Test was used to evaluate significance in tumor growth-rate inhibition, and survival was analyzed using the Log-Rank test.

**Results:** The IC50 values of both gemcitabine and cladribine were found to be in nM concentrations for all cell lines tested, consistent with previous literature values. Cladribine resulted in significant effects on cell death in one EGFRvIII-expressing cell line, while there were no significant effects on cell death with gemcitabine in our experiment. However, gemcitabine resulted in significant effects on cell invasion in all EGFRvIII-expressing GBM cell lines tested. Interestingly, increasing concentrations of gemcitabine resulted in decreased invasion in one cell line and increased invasion in another cell line. No effect was seen with gemcitabine on *in vivo* tumor growth-rate inhibition. While tumor growth-rate inhibition in mice treated with cladribine compared to control mice was not quite statistically significant (p=0.1198), results were promising especially when considered in the context of the low sample size (n=5) for each treatment group. With both gemcitabine and cladribine, there were no significant effects on survival.

**Conclusions:** Ultimately, we concluded that as a drug monotherapy, gemcitabine is not a good option for treating GBM. However, with our promising results on tumor growth-rate inhibition in mice models, we conclude that cladribine warrants further research as a potential agent in GBM.
Development of a Clinical Decision Tool and Protocol for Identification and Treatment of Patients With Corticosteroid Induced Hyperglycemia

Morgan Simons, Joseph Futoma, Kristen Corey, Michael Gao, Marshall Nichols, Krista Whalen, Mark Sendak, Finale Doshi-Velez, Ann McGee, and Tracy Setji

Duke Institute for Health Innovation Clinical Research and Innovation Scholarship

Background: Patients who receive high dose corticosteroids are at increased risk of developing sustained hyperglycemia and adverse effects, including mortality. There is currently no established workflow or protocol to identify and treat these patients in the hospital setting.

Methods: Various machine learning approaches were utilized to determine if a model could predict which patients receiving high dose corticosteroids would develop sustained hyperglycemia. A cohort of 11995 inpatients seen at Duke University Hospital (DUH) between Oct 2014 and Aug 2018 was selected. Patients in this cohort were ≥ 18 years old and received high dose corticosteroids (≥ 20mg/day of prednisone equivalents). Thirty-two features were chosen based upon the expertise of an interdisciplinary team including an inpatient endocrinologist and pharmacist. The outcome of hyperglycemic events was defined as patients having two blood glucose values above 180 mg/dL within 12 hours of corticosteroid administration. The models were evaluated using k-fold cross validation on data from DUH and validated on patient data sets from Duke Regional Hospital (DRH) and Duke Raleigh Hospital (DRAH). Model performance was evaluated using AUROCs and AUPRCs. A clinical workflow was developed to work in tandem with the model in clinical practice.

Results: The gradient boosted model performed significantly better than other models with an AUROCs of 0.872, 0.902, and 0.871 and AUPRCs of 0.524, 0.686, and 0.584 at DUH, DRH, and DRAH respectively. Tables 1 and 2 demonstrate the results for other models.

Conclusions: The gradient boosted model outperformed other machine learning techniques to successfully predict which patients were at risk of corticosteroid-induced hyperglycemia. The performance of the model across hospitals demonstrates the applicability of this approach at other institutions. The clinical workflow and model are currently being piloted at DUH on the Bone Marrow Transplant Unit.

Table 1: AUROCs of Models Across Hospitals (DUH, DRH, DRAH)

<table>
<thead>
<tr>
<th>Models</th>
<th>Training Set</th>
<th>Validation Set 1</th>
<th>Validation Set 2</th>
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<tbody>
<tr>
<td></td>
<td>AUROC (95% CI)</td>
<td>AUROC (95% CI)</td>
<td>AUROC (95% CI)</td>
</tr>
<tr>
<td>Logistic Regression</td>
<td>0.834 (0.828, 0.839)</td>
<td>0.893 (0.892, 0.894)</td>
<td>0.856 (0.855, 0.862)</td>
</tr>
<tr>
<td>Ridge Regression</td>
<td>0.881 (0.879, 0.883)</td>
<td>0.885 (0.884, 0.887)</td>
<td>0.856 (0.855, 0.862)</td>
</tr>
<tr>
<td>Elastic Net Regularization</td>
<td>0.861 (0.857, 0.865)</td>
<td>0.895 (0.894, 0.896)</td>
<td>0.863 (0.861, 0.865)</td>
</tr>
<tr>
<td>Lasso Regression</td>
<td>0.862 (0.857, 0.866)</td>
<td>0.896 (0.893, 0.898)</td>
<td>0.865 (0.863, 0.867)</td>
</tr>
<tr>
<td>Gradient Boosting</td>
<td>0.872 (0.869, 0.876)</td>
<td>0.902 (0.900, 0.903)</td>
<td>0.871 (0.870, 0.872)</td>
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Table 2: AUPRCs of Models Across Hospitals (DUH, DRH, DRAH)

<table>
<thead>
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<th>Models</th>
<th>Training Set</th>
<th>Validation Set 1</th>
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<td></td>
<td>AUROC (95% CI)</td>
<td>AUROC (95% CI)</td>
<td>AUROC (95% CI)</td>
</tr>
<tr>
<td>Logistic Regression</td>
<td>0.471 (0.459, 0.483)</td>
<td>0.567 (0.564, 0.569)</td>
<td>1.530 (1.526, 0.544)</td>
</tr>
<tr>
<td>Ridge Regression</td>
<td>0.481 (0.479, 0.494)</td>
<td>0.590 (0.577, 0.602)</td>
<td>1.557 (1.553, 0.560)</td>
</tr>
<tr>
<td>Elastic Net Regularization</td>
<td>0.483 (0.482, 0.494)</td>
<td>0.671 (0.668, 0.683)</td>
<td>1.569 (1.567, 0.572)</td>
</tr>
<tr>
<td>Lasso Regression</td>
<td>0.494 (0.481, 0.504)</td>
<td>0.672 (0.669, 0.677)</td>
<td>0.571 (0.568, 0.575)</td>
</tr>
<tr>
<td>Gradient Boosting</td>
<td>0.524 (0.512, 0.537)</td>
<td>0.686 (0.680, 0.692)</td>
<td>0.584 (0.580, 0.589)</td>
</tr>
</tbody>
</table>
Silencing the endogenous immune response to tumor implantation

Andrew Barbour, PhD, Steven Shen, MS, Kelly Hotchkiss, PhD, Luis Sanchez-Perez, PhD, John Sampson, MD, PhD, MBA

Stead Scholarship Program of the Duke University Department of Medicine

**Background:** Models testing preclinical efficacy of immunotherapy often rely upon tumors surgically implanted into murine hosts. Recently, the preclinical synergy of immune checkpoint inhibition and radiation therapy in a murine model was found to be an artifact of surgical tumor implantation, and anti-CD40L inhibited this tumor-implantation artifact. Here, we asked if intracranial (IC) implantation of CT2A tumors created an endogenous immune response affecting a model system of CAR-T cell immunotherapy for glioblastoma multiforme. To accomplish this, we conducted two experiments: (1) determining how anti-CD40L given at time of tumor implantation affects the tumor host’s immune phenotype; and (2) establishing a positive control for adoptive transfer of tumor immunity, which could then be mechanistically abrogated via anti-CD40L.

**Methods:** In experiment 1, n=10 C57BL/6 mice underwent IC implantation with CT2A-EGFRvIII tumors. Of these, n=5 were treated with anti-CD40L to inhibit immune costimulation at time of tumor implantation. Tumor infiltrating lymphocytes (TILs) and cervical lymph nodes were harvested for flow cytometry quantification of CD4 and CD8 phenotypes. This experiment was conducted twice, once 7-days following tumor implantation, and once 14-days following implantation. In experiment 2, splenocytes were harvest from n=5 naïve C57BL/6 mice and n=5 mice following IC implantation of CT2A-EGFRvIII tumors. The splenocytes were adoptively transferred into naïve hosts, and the hosts underwent IC implantation of parental CT2A tumors. Survival was estimated via the Kaplan-Meier method.

**Results:** In experiment 1, quantification of TILs was complicated by technical limitations, while absolute counts of CD4 and CD8 in the cervical lymph nodes were lower in the anti-CD40L treatment group. In experiment 2, there was no significant difference in survival between mice receiving adoptive transfer of splenocytes from naïve hosts as compared to hosts implanted with CT2A-EGFRvIII tumors.

**Conclusions:** Inability to adoptively transfer endogenous tumor immunity is suggestive of global immunosuppression caused by intracranial tumor implantation. Technical limitations will be addressed to appropriately immunologically phenotype IC CT2A-EGFRvIII tumors +/- anti-CD40L. Future work will directly apply anti-CD40L to tumor implantation prior to experimentation with CAR-T cells, to determine if the curative ability of CAR-T cell therapy in preclinical GBM models is reliant upon endogenous immunity created by tumor implantation.
Understanding the Biased Signaling of G-Protein Coupled Receptors: A CXCR3 Phosphorylation Barcode

Dylan Eiger, Jeffrey Smith, Tujin Shi, Chia-Feng Tsai, Jon M. Jacobs, Rachel Glenn, Jaimee Gundry, Ronnie Shammas, Issac Choi, Richard D. Smith, Sudarshan Rajagopal

Supported by the National Institute of General Medical Sciences of the National Institutes of Health under Award Number 5T32GM007171-44

Background: G-Protein Coupled Receptors (GPCRs) are the largest and most diverse family of receptors found in eukaryotic organisms. Upon receptor activation, heterotrimeric G proteins exchange GDP for GTP, leading to receptor dissociation and subsequent activation of downstream G protein dependent signaling cascades. The C-terminus of the receptor is then phosphorylated in a specific pattern, or barcode, leading to the recruitment of β-arrestin, steric inhibition of G protein recruitment, and initiation of β-arrestin dependent signaling. It is now appreciated that some ligands are biased towards activation of G protein over β-arrestin dependent signaling, or vice versa. To investigate the mechanisms and implications of GPCR bias, we studied CXCR3, a chemokine receptor present on lymphocytes, which primarily binds three different endogenous ligands: CXCL9 (MIG), CXCL10 (IP-10), and CXCL11(I-TAC).

Methods: To evaluate the function of the phosphorylation barcode, we transfected HEK293N cells with different luciferase tagged CXCR3 constructs with C-terminal mutants at potential phosphorylation sites, and fluoresently tagged β-arrestin-2 and used bioluminescence resonance energy transfer (BRET) to measure β-arrestin-2 recruitment to the receptor. Similarly, we evaluated G-protein activation of the CXCR3 mutants using a TGF shedding assay. To determine how ligand bias leads to changes in downstream signaling pathways, we transfected HEK293N cells with CXCR3 and stimulated them with vehicle control or the previously described ligands. We used mass spectrometry and an antibody-free strategy involving high-pressure, high-resolution separation coupled with intelligent selection and multiplexing (PRISM) for quantification of phosphorylated and non-phosphorylatedproteins.

Results: BRET and TGF assays of the CXCR3 mutants stimulated with CXCL11 demonstrated that C-terminal truncation mutants greatly enhance ligand induced G protein activation while simultaneously abrogating β-arrestin recruitment. Additionally, the S245A mutation significantly enhances CXCL11 dependent β-arrestin recruitment while mutations at T360A/S361A/S364A/S366A reduce this recruitment. Mass spectrometry revealed unique phosphorylation profiles of HEK293N cells across 19362 phosphosites, 1524 of which showed statistically significant differences in levels of phosphorylated protein between the four treatment conditions after five minutes. Gene Ontology and Kyoto Encyclopedia of Genes and Genomes analyses demonstrated that the observed differential regulation of the phosphoproteome includes proteins involved in cell-cell adhesion, transcription and mRNA processing, cytoskeletal organization, and covalent chromatin modifications.

Conclusions: We demonstrated that there are specific phosphorylation sites on the C-terminus of CXCR3 which have functional roles in promoting or abrogating β-arrestin recruitment. Additionally, we showed that activation of the GPCR CXCR3 by three of its endogenous ligands leads to rapid and drastically different phosphoproteomic profiles. Future studies include determining the specific phosphorylated residues in the CXCR3 phosphorylation barcode after ligand stimulation, and further understanding of the physiologic role ligand bias has on chemokine receptor function.
Tissue Factor: A Protein Kinase C-Specific Orchestration

Celia Reynolds, Laura J. Sommerville, Phoebe Xu, Maureane Hoffman

Hackel Fellowship (Department of Pathology. DUSoM), 2019 NASTH/HRT Research Fellowship

**Background:** Tissue Factor (TF) is a critical component of coagulation that also participates in vascular development, angiogenesis, cellular signaling and migration; however, high expression of TF is associated with a wide range of pathologies including malignancy and metastasis. Constituting the first known demonstration of physiological downregulation of TF, a dramatic reduction in baseline TF expression by perivascular cells (pericytes) was recently identified during angiogenesis at sites of wound healing. Further elucidation of this novel mechanism showed that the loss of TF in pericytes exposed to phorbol esters, known inducers of angiogenesis, occurs in a Protein Kinase C (PKC)-dependent manner. Further highlighting the importance and individuality of this distinctly unique phenomenon, the literature to date shows that PKC mediates an increase of TF in all other cell types. There are currently no strategies to mediate TF expression. While the role of diminished TF in pericytes in normal wound healing and angiogenesis is not yet fully defined, mechanistic elucidation poses the potential to serve as a therapeutic approach in the numerous pathologies associated with aberrations of this control. In this study, we aimed to clarify mechanisms underlying the unique pattern of TF expression in pericytes through a comparative study with smooth muscle cells.

**Methods**- Utilizing a range of known activators and inhibitors of PKC, we characterized TF transcription and protein expression in primary cultures of human pericytes and aortic smooth muscle cells (AoSMCs) to characterize the PKC-dependent and isoform-specific nature of TF control in these cell lines. Smooth muscle cells were chosen for use in this model as recognized expressers of TF that are also closely related to pericytes in regards to their mesenchymal lineage and phenotypically different in the setting of injurious or inflammatory insult.

**Results**- Exposure to phorbol 12-myristate 13-acetate (PMA) resulted in opposing effects on TF modulation in these closely related cells that appears to occur in a PKC-dependent manner. PMA induced increased synthesis of TF mRNA and protein expression in AoSMCs, whereas pericytes experienced significant and prolonged downregulation. Accordingly, inhibitors of PKC attenuated the above response to phorbol esters in pericytes; however, augmented TF upregulation in AoSMCs. Furthermore, these inhibitors alone prompted an upregulation of TF similar to that seen with PMA treatment in AoSMCs. Preliminary investigation into the role of p38 in PKC-dependent modulation of TF yielded the most striking upregulation of TF in AoSMCs when administered in combination with PMA.

**Conclusions**- The observed differences in the modulation of TF in PKC-interrogated pericytes and AoSMCs is consistent with varied cell-type specific expression and function of PKC isoforms. Based on the expression profiles of each cell type and isoform-specific affinity of our reagents, we believe PKC-a could be responsible for downregulating TF in both cell-types, despite vastly different constitutive expression. It is unclear how exactly upregulation occurs; however, we hypothesize that modulation requires simultaneous activation of PKC and other signaling molecules such as p38. Thus, further understanding the specific role of PKC isoforms in the modulation of TF has the potential to identify therapeutic targets that can be utilized in the treatment of a wide range of human disease.
Neutrophils Impact Therapeutic Response of Undifferentiated Pleomorphic Sarcoma

Hong CS, Wisdom AJ, Cooper DE, Kuo HC, Mowery YM, Xu E, Luo L, Ma Y, Williams N, Kirsch DG
Poindexter Basic Science Scholarship (2017-2018) and the Eugene A. Stead Research Scholarship (2018-2019)

Background: Undifferentiated pleomorphic sarcoma (UPS), the most commonly diagnosed adult soft tissue sarcoma, present in the extremity are treated with local radiotherapy before or after surgery. Despite high rates of local control, approximately half of high-risk sarcoma patients develop metastases and have low survival rates. Neutrophils, which are present in the microenvironment of most solid tumors, have been shown to independently predict poor patient survival. This includes patients who receive curative-intent radiation therapy, which can recruit myeloid cells to tumors, promoting tumor growth, immune evasion, and radiation resistance. In addition, our preliminary results showed that the depletion of the immune system had a significant effect on tumor growth after radiation.

Methods: To test the role of neutrophils to radiation response of autochthonous tumors, we used immune cell lineage tracing in a genetically engineered mouse model (GEMM) of UPS localized in the extremity using FlpO-recombinase. We then used dual recombinase technology with Cre recombinase to genetically deplete the MRP8+ lineage of neutrophils. We assessed vasculature (CD31) using histology and tumor morphology with H&E. We also did a pharmacological depletion of neutrophils using an in vivo Ly6G antibody injected intraperitoneally every 3 days for 12 days. To test a T-cell independent mechanism, we used an in vivo CD3 antibody injected intraperitoneally to deplete T-cells in autochthonous tumors. To verify the presence of absence of cell populations, we used flow cytometry to quantify the appropriate cell markers.

Results: Using a tdTomato reporter and flow cytometry, we confirmed the MRP8+ promoter in the GEMM targeted neutrophils. Genetically depleting MRP8+ lineage of neutrophils improved the radiation response (20Gy) in both growth and overall survival of our autochthonous UPS tumors. However, it did not make a difference on tumor growth in the cohort treated with 0 Gy. There was no significant difference in CD31+ and tumor morphology on histology. Similar tumor growth and overall survival effects were seen in the neutrophil antibody-depleted cohorts. To assess the role of T-cells, we depleted T-cells in mice treated with 20 Gy and saw no significant difference in tumor growth and survival.

Conclusions: Neutrophil depletion prior to radiation therapy led to an improved radiation response of UPS in both genetic and pharmacologic approaches. This effect is not mediated by changes in tumor morphology or vasculature. We also concluded this effect was mediated by a T-cell independent mechanism. Future studies are needed to address the mechanisms by which neutrophils promote radiation resistance in vivo.
Exosomal Immunomodulator Expression as a Prognostic Indicator in Non-small Cell Lung Carcinoma and Ovarian Carcinoma Patients

Tristan Lee, Ryan Bushey PhD, Edward Patz MD

Background: The association between the presence of complement factor H autoantibodies and early-stage lung cancer has been previously documented, identifying a potential avenue for targeted immune therapy via activation of the complement system and complement-mediated cytotoxicity of tumor cells. At the same time, immune system markers isolated on microvesicles have been identified as key contributors to the processes of disease progression and metastasis, playing an important role in the spread of neoplastic disease. Identifying trends in the serum expression levels of these, and similar immunomodulator markers, would help untangle the processes by which exosomes and like microvesicles facilitate disease progression and identify potential targets for prognostic assessment of disease burden.

Methods: We interrogated a cohort of non-small cell lung cancer and advanced ovarian carcinoma patients for quantitative levels of CFH and other immunomodulatory markers’ expression in isolated microvesicles, with the goal of elucidating the mechanism by which the presence of CFH autoantibodies and the process of neoplastic disease progression interact, using clinical outcomes as a proxy. This process was accomplished via isolation of microvesicles from patient sera samples through ultracentrifugation and exosome precipitation, with concomitant protein quantitation of autoantibody levels for exosomal CFH (and additional immune markers) using traditional western blotting techniques.

Results: We performed analyses across a broad spectrum of categorizing variables, including patient tumor staging, presence of post-treatment recurrent disease, presence of CFH autoantibody, etc., but were unable to identify a clear association between any of these variables and the quantitative levels of CFH identified in patient samples. Analyses of additional immunomodulator markers demonstrated similar results, in samples from patients with non-small cell lung carcinoma and ovarian carcinoma.

Conclusions: Based on the results of our study, we conclude that no significant association between the levels of CFH (or PDL-1, CD-55, CD-59) in patients with non-small cell lung cancer or ovarian carcinoma and the clinical outcomes of these patients can currently be assessed. Future inquiry into this topic that addresses the limitations of our work will help characterize the mechanisms by which metastasis and neoplastic disease progression occur and the role exosomal particles play in facilitating these processes.
Incidence of Comorbidities on Death Certificate Data in Women with Gynecological Cancers

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Background: Gynecological (GYN) cancer remains a major source of morbidity and mortality in the United States, and comorbidities are known to play a significant role in survival for women with GYN cancers. Given the known importance of comorbidities to a cancer diagnosis, including effects on treatment options and prognosis, we wished to determine comprehensively the contributors to mortality in order to target preventative measures across an entire population. This project was undertaken to quantify the comorbidities of all women in the U.S. that died after diagnosis of GYN cancer using death certificate data.

Methods: Using CDC WONDER Database death certificate data, all women who died of cancer in the United States from 2003-2016 were included if a cancer diagnosis was listed as a primary or contributing cause of death. Cases were divided into GYN cancer or not, and then as cervical, ovarian, uterine, or other GYN cancer. Other causes contributing to death (comorbidities) were then captured via ICD10 codes and grouped into similar disease states. Chi-squared and Mann-Whitney U tests compared comorbidity incidences between cohorts. Univariate and multivariate binary logistic regressions tested associations between common comorbidities and GYN cancer subgroups while accounting for demographic variables.

Results: Of 35,324,091 death certificates from 2003-2016, 4,177,823 deaths were women with cancer listed as a cause of death. 440,792 were GYN cancer, with cervical cancer in 62,807; uterine cancer in 134,420; ovarian cancer in 221,119; and other GYN cancers in 25,946. Women with GYN cancers were more likely to have intestinal obstruction (Odds Ratio [OR] 4.05), ascites (OR 3.30), HIV (OR 1.53), kidney disease (OR 1.33), and thrombo-embolic disorders (OR 1.25) when compared to non-GYN cancers (all p-values < 0.000001). Uterine cancer was more likely to have diabetes (OR 1.75), metabolic syndrome (OR 1.60), thrombo-embolic disorders (OR 1.48), ischemic heart disease (OR 1.43), than other GYN cancers (all p-values < 0.000001). Cervical cancer was more associated with HIV (OR 10.73), tobacco-related disorders (OR 2.56), and kidney disease (OR 1.56) (all p-values < 0.000001). Ovarian cancer was more likely to have ascites (OR 2.93), intestinal obstruction (OR 2.04), and pleural effusion (OR 1.92) (all p-values < 0.000001). Multivariate analysis indicated that the associations between the most common comorbidities noted and GYN cancer subtype remained significant when accounting for demographic variables including age, education level, race, ethnicity, and marital status.

Conclusion: In this comprehensive analysis of cancer and medical comorbidity in women in the U.S., comorbidities significantly differ between women who died of GYN cancers versus non-GYN cancers, as well as between GYN cancer subtypes. This has relevance to preventive and therapeutic strategies that may be differentially employed to reduce morbidity and mortality, augmenting cancer treatment. In particular, women with uterine cancer may benefit from reduction in risk of diabetes, ischemic heart disease, and thrombo-embolic disorders; those with cervical cancer may benefit from smoking cessation and optimal HIV management if coincident.
How Well do Barium Swallow Findings Correlate with Esophageal Function Tests in Lung Transplant Candidates?

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**Background:** Esophageal dysmotility and gastroesophageal reflux disease (GERD) are common in patients with advanced lung disease (ALD) and their presence is associated with adverse outcomes after transplantation. Consequently, pre-op testing to assess lung transplant candidacy is common practice. No consensus exists to guide testing; as a result, patients may undergo a variety of tests including ambulatory pH monitoring (pH-metry) and esophageal high-resolution manometry (HRM), which are considered the gold standard for testing esophageal function. Barium swallow (BaS) is a potentially useful adjunct to testing given its non-invasive nature and ability to provide a gross estimate of aspiration as well as identify esophageal contractility/motor disorders, reflux, and luminal irregularities. Thus far, studies in non-ALD patients have been inconsistent regarding the correlation between esophageal function tests (EFTs) and BaS. Therefore, we aimed to assess the reliability of BaS findings in lung transplant candidates compared to formal EFTs. The secondary aim was to identify the prevalence of esophageal anatomic abnormalities in this ALD population.

**Methods:** We performed a retrospective cohort study of ALD patients undergoing evaluation for lung transplantation between 2015 and 2016 at Duke University Medical Center. Per institutional protocol, all patients have HRM, pH-metry, and BaS independent of clinical history or symptoms. Baseline demographic data, reported symptoms, and results of EFTs and BaS were collected. Appropriate statistical tests were performed to evaluate the relationship between EFTs, BaS findings, and patient symptoms.

**Results:** 226 patients underwent EFTs during the study period. Abnormal motility was reported in 59% by BaS and 56% had any abnormal diagnosis on HRM. Upon subset analysis, there was a significant difference in the marginal proportions between the two tests (p < 0.01) with only fair agreement (κ= 0.23). Tertiary contractions on BaS were not associated with HRM diagnoses. Normal passage of a barium tablet was associated with normal motility on HRM (p < 0.01), though 26% of patients with abnormal tablet passage had normal HRM. Mean baseline lower esophageal sphincter pressure was associated with the presence of reflux on BaS (p=0.02). However, there were significant differences in the detection of GERD between modalities with BaS having poor sensitivity (35%) and specificity (77%). There was no meaningful agreement between the presence of dysphagia and dysmotility on BaS (κ= 0.05) or reflux symptoms and GERD on BaS (κ= 0.05).

**Conclusions:** Consistent with prior studies in the non-transplant population, BaS results do not correlate well with formal EFTs in patients with ALD. Although normal tablet passage was associated with normal motility, neither symptoms, the presence of tertiary contractions, nor reflux on BaS were predictive of clinically meaningful findings. Routine BaS as part of a lung transplant evaluation may not add value to formal EFTs and its inclusion in the workup of ALD patients should be further assessed.
Optimization of a Phantom for Quantitating Respiratory Motion Artifacts
Encountered during Helical CT: Conception, Design, Building, and Testing

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**Background:** Computed tomography (CT) is an important investigative tool in medicine that must undergo acceptance testing to ensure that it is reliable and reproducible. Imaging phantoms are artificial models designed to mimic a variety of conditions in humans. The use of phantoms to determine optimal scan parameters is a well-established method that enables clinical investigators to conduct research without exposing patients to repeated doses of ionizing radiation. The purpose of this study was two-fold: [1] construct an affordable neonate imaging phantom capable of simulating respiration using Fusion Deposition Modeling (FDM) 3D printing technology and [2] determine optimal scan parameters for respiring neonatal patients such that image quality is preserved without breath holds. Thus, we hypothesized that there is an optimal scan parameter at which the difference in motion artifact in a motion phantom versus a stationary phantom will be negligible.

**Methods:** The imaging phantom was designed using FreeCAD and Autodesk Fusion 360 software and produced at onsite 3D-printing facilities. Using data on the average chest circumference of neonates, the phantom was designed to approximate 354 mm in circumference with a wall thickness of 4mm. The phantom consists of two nearly identical cylinders, with asymmetry at the top to reflect anatomical differences of the diaphragm. Two 4mm spheres positioned at differing points atop the phantom diaphragm represent lung abnormalities detectable by CT. In order to reproduce diaphragmatic motion, the phantom was designed to attach to a DIY linear actuator programmed via Arduino software to simulate continuous respiration. Preliminary testing involved manual motion of the phantom. The phantom was scanned at various table speeds using a GE Revolution scanner. Finally, Hounsfield units (HU) were measured in a defined region of interest (ROI) of roughly 1.8 x 1.8mm for the phantom at rest (phantom A) and in motion (phantom B) to characterize the severity of motion artifact.

**Results:** Based off of our production costs, the 3D printed phantom is an estimated 86% cheaper than commercial phantoms on the market, making it a reasonable option for further radiology or radiation oncology research. Preliminary testing showed that phantom B has less HU on average than phantom A with an average of 54.36 HU in comparison to 86.77 HU; however, these values are not statistically significant likely due to small sample size.

**Conclusion:** Our findings suggest that 3D-printed, anthropomorphic phantoms may be used to establish a threshold for table speeds above which breath-holding is no longer required for CT imaging. The preliminary results will be expanded to include the use of our linear actuator set at varying speeds, an expansive set of scan parameters, and different CT scanner models.
Predicting upstaging of DCIS to invasive disease: influence of radiologic and pathologic features on radiologist’s performance

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Background: Approximately 20% of DCIS diagnosed at core needle biopsy is upstaged to invasive cancer on surgical excision. Pathologic and clinical information can influence core biopsy classification, but radiologist assessed features have been incompletely assessed. Identification of invasive disease at the time of core biopsy has important implications for clinical management. The purpose of this study is to quantify breast radiologists’ performance at predicting upstaging of DCIS to invasive disease and to identify radiologic and pathologic features that influence performance.

Methods: Mammographically detected calcifications that were initially diagnosed as DCIS on core biopsy, and underwent definitive surgical excision between 2010 and 2015 were identified. 30 cases of suspicious calcifications upstaged to invasive ductal carcinoma and 120 cases of DCIS confirmed at the time of definitive surgery were randomly selected. Nuclear grade, estrogen and progesterone receptor status, patient age, measurement of long axis and breast density information was collected. Ten breast radiologists independently reviewed these cases and estimated the likelihood that the lesion would be upstaged to invasive disease at surgical excision. A binary classifier was created combining both clinical and radiologist’s assessment and trained using a logistic regression.

Results: The mean AUC performance to predict invasive disease was 0.620 (95% CI: 0.489-0.751). Readers had improved performances identifying invasive tumor when evaluating lesions <2 cm (AUC: 0.676 vs 0.500; p = 0.002). Exclusion of micro-invasive cases resulted in significant increase in performance (AUC: 0.651 vs 0.620; p = 0.005). The 100 iteration, 5-fold cross-validation logistic regression had a mean AUC of 0.547.

Conclusion: Radiologists were able to predict invasive disease with moderate accuracy, particularly for smaller DCIS lesions (<2 cm) and after the exclusion of microinvasive disease.