IMPRS Honorable Mentions

Intravital Microscopy Optimization for Murine Tail Lymphedema Model

Diaz S, Mohan G, Khan I, Sinha M, Sen CK, Hassanein AH

Background: Lymphedema is limb swelling caused by lymphatic dysfunction. It occurs in 30% of patients that undergo axillary lymph node dissection in the treatment of breast cancer. It can cause pain, impair function, and decrease quality of life. Lymphedema is treated with compression, excisional procedures and microsurgical physiologic procedures. There is no cure for this disease. The murine tail model of lymphedema is an established animal model for lymphedema. Visualization of lymphatics and functional assessment remains a challenge.

Project Rationale: Immunohistopathology and qRT-PCR are two commonly used in vitro techniques for molecular assessment of lymphatics in animal tissues. These methods provide incomplete information about the structure/function of lymphatics and introduce the confounder of harvested tissue. Methods of functional evaluation such as lymphoscintigraphy or lymphangiography show transit of dyes through lymphatics without high resolution imaging of the lymphatic vessels. Intravital two-photon microscopy (IVM) addresses these disadvantages through real-time imaging of subcellular level biological processes in live animals. The goal of this project is to optimize IVM methods for the assessment of functional lymphangiogenesis in the murine tail lymphedema model.

Methodology Development: A full-thickness skin excision is performed near the base of the tail in C57BL/6 mice. The lymphatic trunks are then surgically transected. Gene-based therapy is delivered to the tail at the surgical site. At 10 days post-treatment, a second full-thickness skin excision is made distal to the site of occlusion. FITC-Dextran (2000 kD) is injected at the distal tail for lymphatic uptake. Lymphatic vessels are visualized at the second skin excision site with the Leica SP8 Confocal/Multiphoton Microscope and assessed for number of branching points. Images are captured with Leica Application Suite Advanced Fluorescence Software and analyzed with Imaris Microscopy Image Analysis Software. This results in the ability of functional assessment of lymphatics and visualization of lymphangiogenesis following gene-based therapy.

Changes in Cortical Composition during Gyrification in the Developing Brain

Doherty S, Garcia KE

Background and Hypothesis: Abnormal brain folding has been implicated in neurodivergent conditions such as schizophrenia and autism, yet the mechanical and biological processes responsible for this process are not well understood. One current hypothesis is that cortex growth outpaces growth of the underlying white matter to drive mechanical buckling. However, mechanical stresses, such as those resulting from buckling, can also influence cellular behavior. In this study, we hypothesized that mechanical stresses from cortical folding influence processes of biological growth within the cortex, such as dendrite arborization within the neuropil and neuronal differentiation.

Methods: To quantify change in cell body size and neuropil over the period of cortical folding, sections of the developing ferret brain (postnatal days 20, 26, 32, and 38) were stained with FluoroNissl dye, imaged with confocal microscopy, and analyzed using Fiji software. Change in percent neuropil, cell area, cell density, and overall length were quantified at upper, middle, and lower thirds of the cortex to assess the influence of bending stresses within gyri and sulci during development.

Results: Preliminary analysis revealed a substantial increase in neuropil over time in the upper layers of the cortex. However, gyral regions expected to experience mechanical tension and increased expansion did not exhibit the hypothesized differences in neuropil or cell size. Though there was an overall increase in neuropil volume fraction and cell body size over time, throughout all layers of the cortex, these factors only accounted for roughly 2/3 of the physical growth quantified throughout these cortical layers.
Potential Impact: Findings indicate that neuropil and cell body expansion are insufficient to fully explain the growth observed during cortical folding. These results highlight a potential role for alternative cellular processes, such as the migration of other cell types into the cortex, to induce cortical growth and folding in gyrencephalic species.

Grounded Practical Theory Analysis of Patient-Provider Communication with Black Women Participating in Breast Cancer Clinical Trials

Okoruwa OP, Ridley-Merriweather KE

Background: Previous literature suggests breast cancer clinical trial participation among Black women has declined in recent years by as much as 35%. Though the literature identifies barriers to participation for this population, little has been studied about how researchers can address these barriers. This study investigates the communication between healthcare providers and Black women to illuminate how providers and researchers can positively influence their perceptions of breast cancer clinical trial participation.

Methods: Fourteen women (n=14) who self-identified as Black, Black American, or African American, were interviewed about their communication experiences with healthcare providers regarding breast cancer clinical trial participation. Each transcribed interview was coded using thematic analysis. Grounded Practical Theory was introduced to give insight into the patient-provider communication needs of Black breast cancer research participants.

Findings: The findings fell into four categories: (1) impressions of participants toward their providers, (2) reflections on the clinical trial recruitment experience, (3) communication relationships with medical and research providers, (4) and cultural aspects of patient-provider communication. One major finding was that an important way women learn about clinical trials is through conversations with their oncologists. However, only 29% of Black women interviewed were informed of their clinical trial by a healthcare provider, suggesting that Black women may not be receiving the information they need to participate in clinical trials.

Conclusion: By understanding existing patient-provider communication typologies, we can improve these methods of communication to increase the interest and participation of Black women in breast cancer clinical trials.

Implications: Clinical trials provide data to healthcare providers about treatment options for breast cancer. If minoritized populations are continually underrepresented in clinical trials, these treatments might not prove to be efficacious in Black women. Researchers must make the necessary investment of resources and effort to better understand the needs of Black women in clinical trial recruitment.

Complications in Burn Patients Following Fluid Over-Resuscitation

Peipert LJ, Kraatz J

Background/Objective: Over-resuscitation of burn patients leads to dangerous edema-related sequelae. The Parkland formula is commonly used to predict fluid requirements in the 24 hours following burn injury, yet studies report widely varying resuscitation rates. This study aims to assess fluid resuscitation practices at Lutheran Hospital and evaluate correlations between resuscitation rates and fluid-overload complications.

Methods: A retrospective chart review assessed fluid resuscitation of 36 adult patients with burns affecting at least 15% total surface body area (TBSA) between May 2020-May 2022 at Lutheran Hospital. Intravenous fluid rates and urine output (UO) were recorded for the first 24 hours of each patient's hospital stay. Complications and mortality were recorded for the entirety of a patient's hospital stay. Patients who received volumes exceeding those recommended by the Parkland formula were placed in the high-volume group whereas patients who received a lesser volume were placed in the low-volume group. Statistical analyses were performed using Microsoft Excel ($p = 0.05$).

Results: The study included 36 patients with an average fluid resuscitation of 4.13 ±2.14 mL/kg/%TBSA in the first 24 hours following hospital admission. Average UO in the high-volume group ($n=14$) was 1.33 ±0.76 mL/kg/hr compared to 0.75 ±0.47 mL/kg/hr in the low-volume group ($n=22$). Fluid complications were more common in the high-volume group (41.7%) compared to the low-volume group (19.0%), but this difference was not statistically significant ($p=0.230$). No difference in mortality was observed ($p=1.000$).

Conclusion: The high-volume group had an average UO exceeding the recommended range (0.5-1.0 mL/kg/hr) and experienced greater rates of fluid-overload complications (pulmonary edema, compartment syndromes, etc.). Due to the small sample size and limited power of this study, the difference in fluid-related complications was not statistically significant.

Clinical Impact and Implications: Physicians should limit fluid volumes exceeding the Parkland formula when resuscitating burn patients to avoid fluid overload sequelae.

Development of PET Tracers of Glutamine Metabolism

Rehman I, Arkin Cs, Knapek E, Snyder S, Schulte M

The labeling of amino acids with positron-emitting radionuclides (such as fluorine-18) has been a widely used approach for the imaging of tumors as it often provides higher diagnostic accuracy than what is observed with [18F]FDG. In particular, PET tracers of glutamine metabolism have garnered significant attention in recent years. O-(2-[18F]fluoroethyl-L-tyrosine (18F-FET) is a promising PET tracer in this regard and is currently under investigation at Indiana University (IU) through an expanded access IND for patients with brain malignancies. Clinical production of 18F-FET at IU previously required the use of HPLC for purification, following the reaction of fluorine-18 with the precursor molecule for FET. While this method has been successful in removing undesirable impurities and byproducts, HPLC significantly increases synthesis time and is a common failure point in the synthesis of FET on our current radiochemistry module. To address this issue, we aimed to deploy a solid-phase-extraction (SPE) method for the purification of FET, thereby eliminating the need for HPLC purification. Several methods for the SPE purification of FET have been previously reported; however, none of these strategies afforded pure [18F]FET on our synthesis module, thus development of new methods was required.

While several tracers capable of measuring different aspects of glutamine metabolism have been evaluated in both preclinical and clinical studies, there are metabolic liabilities that limit their utility and complicate data analysis. [18F]-4F-glutamine is one such tracer that has shown promise but has limitations due to undesirable metabolism in vivo. Herein we report our progress towards an improved synthesis of [18F]FET for ongoing clinical studies as well as our progress towards the development of a novel tracer that would