Multidisciplinary Benign Urology Research Symposium

April 20, 2023

Trent Semans Center, Duke University

Duke Multidisciplinary K12 Urologic Research Career Development Program (KURe)

Duke UNIVERSITY
KURe

Urologic Congenitalism and Development: Impact from Prenatal to Adult Life

Trent Semans Center, Duke University,

Durham, North Carolina

Thursday, April 20, 2023

8 am - 4:30 pm

Duke University School of Medicine

8:00 am **CHECK IN: Obtain the link to the program booklet**

8:15 am **WELCOME AND INTRODUCTIONS:**
Cindy L. Amundsen, MD, KURe PI and Program Director

8:25 am **INVITED SPEAKERS AND PANEL DISCUSSION**
Moderators: Austin Livingston, MD, Duke University
Alexandria Spellman, MD, Duke University

8:30 am **Sunder Sims-Lucas, PhD**
Associate Professor of Pediatrics, University of Pittsburgh
Relating maternal health and nutrition preconception and during pregnancy to fetal renal development

8:45 am **Lori O'Brien, PhD**
Assistant Professor, Department of Cell Biology and Physiology University of North Carolina Chapel Hill
Susceptibility to renal disease: from genetics to external factors during fetal development

9:00 am **Alison Sanders, PhD, MS**
Assistant Professor, Department of Environmental and Occupational Health, University of Pittsburgh
Environmental chemicals and kidney function in pregnant women and children

9:15 am **Christina Ching, MD**
Associate Professor, Urology, Nationwide Children's Hospital
Novel biomarkers of urinary tract obstruction

9:30 am Moderated discussion (20 minutes)

9:50 am **POSTER SESSION-1 (ODD NUMBERED POSTERS) AND REFRESHMENTS**

10:50 am **TRAINEE PLATFORM PRESENTATIONS**
Moderator: Mary Barbee, PhD, Temple University

10:55 am **Top Basic Science Abstract:** Michael Odom, PhD, Duke University
Underactive bladders from type 1 diabetic Akita female mice exhibit an increase in contractility via FP receptor activation as a result of NLRP3-mediated inflammation

11:10 am **KURe Scholar:** Em Abbott, PhD, Duke University
Acute and sub-acute effects of CN-105 on bladder function following spinal cord transection in urethane-anesthetized rats

11:25 am **Top Clinical Science Abstract:** Robert Medairos, MD, Duke University
The impact of single use cystoscopes on clinical time workflow in an outpatient setting

11:45 am **LUNCH**
OR
CONVERSATIONS and LUNCH WITH THE EXPERTS at assigned tables
(Pre-registration required)

12:45 pm **POSTER SESSION-2 (EVEN NUMBERED POSTERS)**

1:50 pm to 4:30 pm See next page

1:50 pm **INVITED SPEAKERS AND PANEL DISCUSSION**
Moderators: Claudia Covarrubias Rosas, MD, McGill University
Gabrielle Grob, BA, Virginia Commonwealth University Health System

1:55 pm **Brandon Lane, PhD**
Assistant Professor of Pediatrics, Duke University School of Medicine
Leveraging multiomics tools to understand mechanisms of CKD

2:10 pm **Christopher Cooper, MD**
Professor of Urology, The University of Iowa
Improved predictive factors of clinical outcomes in children with vesicoureteral reflux

2:25 pm **Jonathan Routh, MD, MPH**
Associate Professor of Surgery, Division of Urology, Duke University
Current controversies and challenges in pediatric vesicoureteral reflux

2:40 pm **Maryellen Kelly, DNP, CPNP, MHSc**
Assistant Professor, Division of Healthcare of Women and Children, Duke School of Nursing
Kids, don't drink the pee!

2:55 pm Moderated discussion (20 minutes)

3:15 pm **TRAINEE PLATFORM PRESENTATIONS**
Moderator: Maryrose Sullivan, PhD VA Boston Healthcare System

3:20 pm **Top Basic Science Abstract:** Byron Hayes, PhD, Duke University
Nerve growth factor drives sensory nerve sprouting and persistent pain after recurrent bladder infection

3:35 pm **KURe Scholar:** Cassandra Kisby, MD, Duke University
Exosome-induced tissue healing in a porcine model of bladder mesh exposure

3:50 pm **Top Translational Science Abstract:** Nicole Diaz, BS Duke University
Aging and the female urinary microbiome: associations between Lactobacilli, menopause, and vaginal estrogen use

4:10 pm **PRESENTATION OF TRAINEE AWARDS AND CLOSING REMARKS**

4:25 pm **Evaluations and Feedback**

4:30 pm **ADJOURNMENT**

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Duke Multidisciplinary **K**12 **U**rologic **Re**search Career Development Program (KURe):
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Christina Ching, MD
Christopher Cooper, MD
Maryellen Kelly, DNP, CPNP, MHSc
Brandon Lane, PhD
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S-01

Relating maternal health and nutrition preconception and during pregnancy to fetal renal development

Sunder Sims-Lucas, PhD
Associate Professor of Pediatrics, University of Pittsburgh

The formation of the kidney is a complex process involving tightly regulated genetic signaling. The goal of kidney formation is to produce functional filtering units known as nephrons. Nephron number can be directly correlated with kidney function and susceptibility to diseases later in life. The maternal environment during pregnancy is highly susceptible to environmental changes and this can ultimately affect the formation of the nephrons leading to alterations in nephron number and kidney function. This talk will highlight the role of maternal factors in formation of the kidney and nephron number, a particular focus will be on the role that maternal diabetes plays in mediating kidney development and nephron endowment and injury susceptibility later in life. The presentation will also discuss the current model systems that mimic human kidney formation and give a perspective as to the state of the field and some key questions to be answered.

S-02

Susceptibility to renal disease: from genetics to external factors during fetal development

Lori O'Brien, PhD
Assistant Professor, Dept of Cell Biology and Physiology University of North Carolina Chapel Hill

Disruptions to proper kidney development during the fetal period significantly increases the risk for renal disease and other complications. As nephrons are the major functional unit of the kidney, understanding the factors which impair their proper formation and function are critical. Genetic perturbations, fetal exposures, and premature birth are all factors which can impair proper nephrogenesis by a variety of means. Our understanding of basic kidney development is crucial to developing interventional treatments which can reduce the risk for kidney disease. In my presentation, I will introduce the basics of kidney development, how disruptions predispose to disease, and cover the most recent advances in this area.

S-03

Environmental chemicals and kidney function in pregnant women and children

Alison Sanders, PhD, MS
Assistant Professor, Department of Environmental and Occupational Health, University of Pittsburgh

The pregnant kidney and developing kidney are uniquely susceptible to environmental exposures compared to the mature adult kidney. Environmental exposure to nephrotoxicants (e.g., metals, air pollution and endocrine disrupting compounds) in the periconceptional and prenatal periods may alter structure and functional changes. Further, cumulative health risks of multiple chemical and non-chemical kidney stressors across the lifespan contribute to later life disease risk and severity. Exposures to environmental chemicals may be preventable and represent potentially intervenable risk factors to preserve kidney health. This talk will highlight methods of environmental chemical assessment, discuss study design and biomarker selection in studies of kidney development and pregnancy, and highlight important research gaps to be addressed.

S-04

Novel biomarkers of urinary tract obstruction

Christina Ching, MD
Associate Professor, Urology, Nationwide Children's Hospital

Pediatric urinary tract obstruction can lead to significant renal dysfunction. Our ability to diagnose urinary tract obstruction currently relies primarily on imaging studies, particularly in very young children. This same population, which can have signs of potential obstruction identified during their prenatal ultrasounds, called hydronephrosis, often have resolution of their initially abnormal imaging findings. Differentiating those patients with initially concerning imaging but in whom their hydronephrosis may improve from those in whom the hydronephrosis might worsen and truly indicate an obstruction can be difficult. Properly identifying which patient belongs in which group is of import as it can alter the need for regular urologic follow up and imaging, which can be laborious, stressful to families, and tax the medical care system. Imaging studies can also be invasive and result in radiation exposure. It would enable better targeted care of patients and ideally improved outcomes on renal function with an earlier diagnosis of significant obstruction. Urinary biomarkers, namely through antimicrobial peptides, are a potential means of augmenting if not replacing current diagnostic methods of evaluating for urinary tract obstruction. Urine biomarkers are enticing due to their generally noninvasive method of collection and potential direct pulse on the health of the different cell types of the urinary tract. There have been fruitful investigations into different urinary peptides that might represent urinary obstruction, with ongoing interest and pursuit to identify predictive markers of clinically significant obstruction.

S-05

Leveraging multiomics tools to understand mechanisms of CKD

Brandon Lane, PhD
Assistant Professor of Pediatrics, Duke University School of Medicine

Advances in omics technology and bioinformatics pipelines are speeding up discoveries of genetic causes and genetic risk factors for different diseases. These advances are driving better phenotypic descriptions, identification of new diagnostic tools, and new therapeutic agents. We illustrate this with genomic discoveries in CAKUT and nephrotic syndrome.

S-06

Improved predictive factors of clinical outcomes in children with vesicoureteral reflux

Christopher Cooper, MD
Professor of Urology, The University of Iowa

All children with vesicoureteral reflux (VUR) are not created equal. VUR is associated with a spectrum of risk. Larger degrees of VUR are associated with increased risk of urinary tract infections (UTIs), renal parenchymal abnormalities, and lower rates of spontaneous resolution. The inequality in risk and outcomes among children with VUR dictates the need for employing different management strategies, ideally based on an accurate assessment of each child's risk. Although multiple factors contribute to risk, historically, grade has been used as the primary factor in assessing a child's risk from VUR. Unfortunately, the commonly used International Reflux Study in Children (IRSC) grading system has poor inter-observer reliability. This poor reliability of grading, along with demonstrated improvement in predictive ability by the use of additional parameters identified on a voiding cystourethrogram (VCUG), led to the hypothesis that the use of more objective and reliable VCUG parameters may either augment or replace the current International Reflux Classification grading system to provide more accurate classification and prediction of clinical outcomes. Several such VCUG parameters will be reviewed in this presentation including the distal ureteral diameter ratio (UDR) and bladder volume at onset of VUR.

S-07

Current controversies and challenges in pediatric vesicoureteral reflux

Jonathan Routh, MD, MPH
Associate Professor of Surgery, Division of Urology, Duke University

This presentation will briefly highlight the history of vesicoureteral reflux treatment, recent trends and controversies, and potential future impacts of those trends.

S-08

Kids, don't drink the pee!

Maryellen Kelly, DNP, CPNP, MHSc
Assistant Professor, Division of Healthcare of Women and Children, Duke School of Nursing

During this session a methodology for detecting the microbiome in low biomass samples will be discussed. A review of what is known about the urinary microbiome in infancy and childhood will be presented as well as results from recent studies looking at difference in the urinary microbiome composition based on age and gender.

Underactive bladders from type 1 diabetic Akita female mice exhibit an increase in contractility via FP receptor activation as a result of NLRP3-mediated inflammation

Odom, Michael R.; Hughes Jr., Francis M.; Pope, NiQuava; Jin, Huixia; Purves, J. Todd

Affiliation: Department of Surgery, Division of Urology, Duke University Medical Center, Durham, NC..

Introduction: Diabetic bladder dysfunction, affecting >50% of diabetics, is driven by bladder inflammation triggered by the NLRP3 inflammasome. Prostaglandin (PG) production in the bladder is dysregulated by inflammation but the inflammatory mechanisms responsible during diabetes, and how this disease affects PG-mediated bladder contractility, is unknown. Here, using the Akita genetic mouse model of Type 1 diabetes that develops bladder underactivity crossbred with NLRP3 null mice, we investigated how NLRP3-driven inflammation impacts PG release and bladder contractility.

Methods: Type 1 diabetic Akita mice were crossbred with $NLRP3^{-/-}$ mice to yield: control/$NLRP3^{+/+}$, diabetic/$NLRP3^{+/+}$, control/$NLRP3^{-/-}$, and diabetic/$NLRP3^{-/-}$ mice. Females were aged to 30 weeks when bladder underactivity is apparent. To measure *ex vivo* PG release, strips of isolated mucosa and detrusors were stretched to release PGE2 and PGF2α, which were quantified using ELISAs (n=4). To assess *ex vivo* smooth muscle contractility, contractile force of intact bladder strips in response to PGE2 and PGF2α +/- FP receptor antagonist AL8810, was measured (n=7-8). FP receptor protein expression was determined in detrusors using western blotting (n=6).

Results Diabetes doubled PGE2 released from the mucosa ($p<0.05$) while reducing release from the detrusor by 67% ($p<0.05$). Surprisingly, no changes in PGE2 mediated bladder contractility were noted. In contrast, diabetes did not change PGF2α release in either the mucosa or the detrusor. However, intact bladder strips from diabetics demonstrated a 33% increase ($p<0.05$) in PGF2α-mediated contractility which was abolished by AL8810. In diabetic detrusors, FP receptor protein expression increased by nearly 400% ($p<0.05$). Diabetics lacking NLRP3, and therefore NLRP3-mediated inflammation, did not exhibit dysregulated PG release, PG-mediated bladder contractility, or increased FP receptor proteins.

Conclusions: Diabetes inversely affects PGE2 release from bladder mucosa and detrusor while not altering PGE2-induced contractions. Surprisingly, release of PGF2α was unaffected by diabetes although contractility to PGF2α, via the FP receptor, was increased - consistent with the increase in FP receptors. These changes were driven by NLRP3-induced inflammation. The increased expression of pro-contractile FP receptors suggests FP receptor agonists may be effective therapies to treat existing underactive diabetic bladder dysfunction.

Funding: NIH-K12 DK100024; NIH-RO1 DK117890

Research area: Diabetes, bladder inflammation, voiding dysfunction / urinary retention

Acute and sub-acute effects of CN-105 on bladder function following spinal cord transection in urethane-anesthetized rats

Abbott, Em[1]; La, Priscilla[1]; Wu, Jonathan[1]; Laskowitz, Daniel[2]; Faw, Timothy[3]; Grill, Warren[1]

Duke University; Biomedical Engineering[1], Neurology[2], Orthopaedic Surgery[3]; Durham, NC

Introduction Spinal cord injury (SCI) causes motor and sensory dysfunction which manifests as paresthesias, paralysis, and neurogenic bladder (NGB) that negatively impact quality of life. Our objective is to improve bladder function in persons living with SCI by investigating novel uses for emerging treatments. One promising, understudied area is the role of apolipoprotein E (APOE: gene; apoE: protein), which is produced in the central nervous system and modulates inflammation and neuroplasticity after SCI. APOE4 polymorphism is associated with poor functional outcomes after neurotrauma and impaired nicotinic receptor function, and these changes could worsen bladder function. Exogenous-delivery of the intact apoE protein is ineffective because it does not cross blood-brain barrier. CN-105 is a 5-amino acid peptide (Ac-VSRRR-NH2), developed at Duke, that crosses blood-brain/spinal cord barriers and mimics apoE protein function in part by blocking α7 nicotinic acetylcholine receptors. For these reasons, we expected it to decrease overactive bladder and promote emptying in rats with NGB resulting from SCI. We hypothesized that CN-105 would reduce neurogenic detrusor overactivity (NDO) and suppress detrusor sphincter dyssynergia (DSD) through reduced external urethral sphincter activity (EUS EMG) during voiding, ultimately increasing the efficiency of bladder emptying.

Methods We conducted terminal acute experiments in urethane-anesthetized female Sprague-Dawley rats to quantify the chronic and acute effects of CN-105 on urodynamic function. Rats (n=6) received spinal cord transection (TX) at T9/T10 followed by CN-105 (2.0 mg/kg) or 0.9% saline treatment at 30 min (intravenous; IV), 3 hours (IV), and every 24 hours (intraperitoneal) for 7 days post-injury. We assessed locomotor function prior to injury, and days 1 and 7 post-injury using the Basso, Beattie, Bresnahan locomotor rating scale (BBB). Collection of body weight and void volumes occurred 2-3 times daily. For acute cystometry experiments, we placed a catheter into the bladder dome to measure pressure and an electromyography paddle on the EUS to measure muscle activity. During a single-fill cystometric trial, we infused the bladder with saline until a bladder contraction voided urine or until overflow incontinence occurred. Following 3 baseline cystometric trials, we performed 3 trials after administration of CN-105.

Results We did not detect changes in BBB scores at 1- or 7-days post-injury ($p>0.05$) indicating consistent injury between groups. CN-105 treatment prevented post-injury weight loss consistent with previous generation ApoE mimetic peptides. In agreement with the literature, SCI rats demonstrated increased bladder capacities (BC; CN105: 11.83 ± 2.00; Saline: 13.69 ± 1.42 mL), decreased voiding efficiency (VE; CN105: 3.31 ± 1.83; Saline: 5.76 ± 1.87%), frequent non-voiding contractions (NVCs: CN105: 8.0 ± 2.48; Saline: 13.25 ± 0.96) vs. intact (BC: 0.82 ± 0.43 mL; VE: 23.34 ± 17.61%; NVC: 1.35 ± 1.02; Kruskal-Wallis $p<0.05$). Post-hoc analysis indicates that extended CN-105 may decrease NDO ($p<0.05$), and acute dosing of CN-105 may decrease bladder capacities in chronically treated rats ($p<0.05$).

Conclusion(s) These data indicate potential for extended and acute CN-105 treatment to alter acute bladder outcomes after SCI; however, more data are needed in transected rats and those with a more clinically relevant contusion SCI. Future research should also consider optimizing treatment strategy (timing, dose, route of administration). In addition, APOE targeted replacement in mice expressing the human E3 or E4 polymorphism could provide further mechanistic insight into the role of APOE in post-SCI bladder dysfunction and identify new avenues for treatment.

Research area Urodynamics, Voiding Dysfunction/Urinary Retention, Neurology

Funding NIH K12DK100024; Duke Institute for Brain Sciences Research Incubator Award

The Impact of Single Use Cystoscopes on Clinical Time Workflow in an Outpatient Setting

Medairos, Robert; Soto-Palou, Francois; Dionise, Zachary; Van Namen, Bailey; Locascio, Rachel; Antonelli, Jodi; Preminger, Glenn; Lipkin, Michael

Department of Surgery, Division of Urology, Duke University Medical Center, Durham, North Carolina.

Introduction: Outpatient Urology procedures are historically performed with reusable equipment, which requires dedicated time from clinical staff to perform cleaning and sterilization related tasks. The single-use cystoscope recently developed by Ambu (Ballerup, Denmark) could optimize clinical staff encounter time in a high-volume outpatient setting. No studies exist evaluating the potential clinical time benefit of a single-use cystoscope, which may improve the ancillary staff workflow and overall clinical productivity. Our aim was to evaluate the change in overall clinical encounter time and clinical capacity after transitioning to single-use cystoscopes in an outpatient clinic setting.

Methods: A single-institution prospective study on two full days of outpatient Urological clinical procedure encounters was performed. Two procedure rooms were staffed by two nurses and one provider for an entire clinic day, where cystoscopy procedures were completed. Encounter times for each portion of nursing care responsibilities were observed and divided into discrete categories, and time spent during each portion of the clinical encounter was recorded. Clinical procedures transitioned from reusable to single-use cystoscope clinical encounters in 03/2022, and a single clinic day was observed for reusable and single use cystoscopy clinics. The differences in overall clinical encounter time and capacity (patient visits per day) before and after the transition to single-use cystoscopy procedure clinics were compared.

Results: A total number of 24 outpatient Urology procedures were performed, producing the same number of cystoscopy procedures on both the reusable and single-use cystoscopy clinic days (n=12). Preliminary cystoscope cleaning and transportation tasks by nursing staff were eliminated when utilizing single-use cystoscopes, which accounted for a significant reduction in clinical encounter time (Table 1). The average total encounter time decreased from 66 minutes to 44 minutes, resulting in a 34% reduction in clinical encounter time after transitioning from reusable to single-use cystoscopes. As a result, overall clinical patient capacity with single-use cystoscopy clinics has since improved from 12 to 21 patients per provider clinic day.

Conclusion: Transition to a completely single-use cystoscopy outpatient procedure clinic improved clinical efficiency, which increased the overall clinical patient volume.

Research Area: Quality Improvement

Table 1. Average Clinical Encounter Time for Each Workflow Category

Encounter Workflow Category	Reusable Cystoscope (min.)	Single-Use Cystoscope (min.)
Preparation of cystoscope tray	4	0
Transportation of cystoscope tray	1	0
Setup of cystoscope	5	5
Prepare sterile processing	5	0
Prepare patient for consent	5	5
Intake and provider consent	17	17
Lidocaine instillation	10	10
Procedure length	3	3
Cystoscope breakdown	2	2
Cystoscope preliminary cleaning	10	0
Alert transportation of cystoscope	2	0
Room turnover	2	2
Total	66	44

Nerve growth factor drives sensory nerve sprouting and persistent pain after recurrent bladder infection

Hayes, Byron W[1]; Purves, J Todd[2]; Hughes Jr, Francis M[2]; Ji, Ru-Rong[3,4,5]; Abraham, Soman N[1,6,7]

[1] Department of Pathology, Duke University Medical Center, Durham, NC, USA; [2] Department of Surgery, Division of Urology, Duke University Medical Center, Durham, NC, USA; [3] Department of Cell Biology, Duke University Medical Center, Durham, NC, USA; [4] Department of Anesthesiology, Duke University Medical Center, Durham, NC, US; [5] Department of Neurobiology, Duke University Medical Center, Durham, NC, USA; [6] Department of Immunology, Duke University Medical Center, Durham, NC, USA; [7] Department of Molecular Genetics & Microbiology, Duke University Medical Center, Durham, NC, USA.

Introduction/Objectives Recurrent urinary tract infection (rUTI) is a significant health burden characterized by frequent UTIs with chronic pelvic pain, urinary frequency, and urgency. Many patients continue to experience symptoms while undergoing standard-of-care prophylactic antibiotics, even in the absence of bacteriuria. Previously, we described a mouse model of rUTI consisting of 3 once-a-week E. coli bladder infections that leads to increased urinary frequency and pelvic sensitivity 14 days after the last infection, when urine cultures are negative. Analysis of the bladders of these rUTI mice revealed significant sensory nerve growth in the lamina propria, a finding we also observed in bladder biopsies from rUTI patients. In addition, we found that rUTI mouse bladders had elevated nerve growth factor (NGF) protein levels that was specifically increased in monocytes and mast cells. Based on this, we sought to accomplish two objectives. First, we determined whether monocyte or mast cell derived NGF could directly contribute to nerve sprouting. Second, we assessed whether NGF could be therapeutically targeted in our rUTI model. We hypothesize that NGF produced from both immune cells plays a major role during rUTI, as elevated NGF is found in rUTI patient urine and NGF is known to mediate nerve growth and sensitization in other ailments.

Methods Monocyte and mast cell cultures were generated from bone marrow of healthy mice and individually used in co-culture experiments with dorsal root ganglia neurons obtained from the L6 and S1 levels of the mouse spine. Microscopic image analysis was utilized to assess neurite growth, with recombinant NGF used as a positive control and tropomyosin receptor kinase A (TrkA) antagonist (GW-441756) used to block NGF mediated signaling. Female 8-week-old C57BL/6 wildtype (WT) or knockout mice underwent rUTI to confirm the role of both immune cells. TrkA inhibition in vivo was performed by treating WT mice with GW-441756 or saline either daily (IP and intravesicular), starting at the second infection, or as two individual treatments 14 days after the third infection. Pelvic sensitivity was assessed via von Frey probing of the abdomen 14 days after third infection and Substance P based immunofluorescence analysis was used to assess bladder sensory nerve growth in each mouse.

Results We found that both monocytes and mast cells each induce NGF dependent neurite growth in cultured neurons; in addition, this nerve growth was prevented in monocyte or mast cell targeting knockout mice after rUTI, supporting the role of NGF from each cell type in nerve sprouting. Confirming this, continuous inhibition of TrkA during rUTI prevented nerve growth and pelvic sensitivity in mice. Lastly, blocking TrkA 14 days after the third infection reversed pelvic sensitivity in mice, suggesting the role of NGF in prolonging sensitization.

Conclusions These studies reveal monocyte and mast cell derived NGF mediates nerve growth during rUTI. In addition, these results present NGF as a potential therapeutic target for relief in rUTI patients. Future work will assess possible roles of other neurotrophins during rUTI.

Research area Infections of the Urinary Tract, Neurourology, Therapeutic Development

Exosome-Induced Tissue Healing in a Porcine Model of Bladder Mesh Exposure

Kisby, Cassandra K.[1]; Farraguna, Sam[2]; Gilpin, Anna[3]; Newman, Hunter[4]; Amundsen, Cindy L.[1]; Varghese, Shyni[3,4,5]

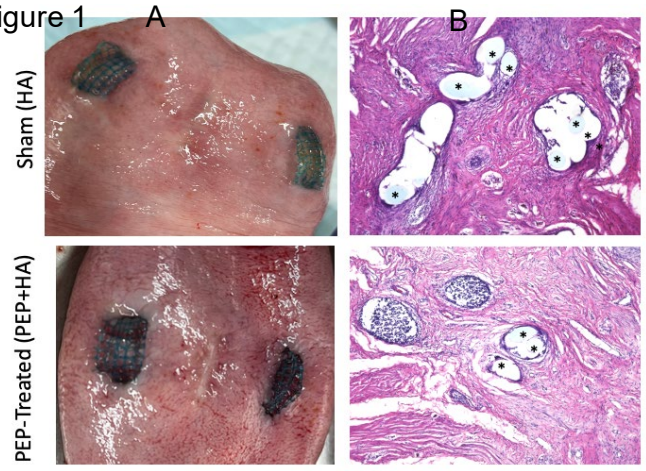
[1]Department of Obstetrics and Gynecology, Division of Urogynecology, Duke University Medical Center, Durham, NC. [2]Duke University, Durham, NC. [3]Department of Biomedical Engineering, [4]Department of Mechanical Engineering and Materials Sciences, Duke University, Durham, NC. [5]Department of Orthopedic Surgery, Duke University Medical Center, Durham, NC.

Introduction: Purified human plasma-based extracellular vesicles offer an acellular, shelf-stable bioactive platform, known as purified exosome product (PEP), which has been shown to augment wound healing without the cost and challenges associated with cell-based technologies. These characteristics make this technology an ideal system for enhancing tissue healing in the setting of pelvic mesh exposure. Prior preclinical studies have demonstrated the efficacy of PEP in promoting tissue regeneration and resolving vaginal mesh exposures following vaginal reconstructive surgery. Given mesh is readily used for anti-incontinence procedures, and a complication of such is bladder mesh exposure, we aimed to explore utilization of this technology in the bladder. The objectives of this study were to explore the utility of an injectable purified exosome product to (1) resolve bladder mesh exposures, as well as (2) evaluate localized histologic changes in treated versus untreated tissues.

Methods: 10 Yorkshire-Crossed pigs were utilized, 6 in the treatment (PEP) group and 4 in the sham group. Through an open abdominal incision, polypropylene mesh arms were deployed by a Urogynecologic surgeon (day 0), intentionally perforating the right and left bladder dome to mimic two mesh sling exposures (1x2cm), Treatment group bladders were injected with PEP mixed in hyaluronic acid (HA). The sham group received injection of hyaluronic acid-only. On day 14, animals were sedated and re-injected under ultrasound guidance. The animals were euthanized 6 weeks after the sentinel injection. Gross examination and histologic parameters on H&E and IHC-stained sections were evaluated. Persistent exposure was reported as an area of exposure in centimeters.

Results: All animals experienced persistent bladder mesh exposures at the 6-week end point. On gross examination, the meshes were found to be protruded into the bladder lumen, rather than remaining flush with the urothelium, with 2 animals experiencing stone formation on the mesh (Fig 1A). The average area of exposure was lower in the PEP-treated group (average 1.18cm (range .35-1.99) compared to sham (1.35cm, range .42-2), with tissue regeneration largely occurring at the mesh-urothelium interface. Histologic analysis is currently underway. Initial evaluation demonstrates higher fibrin deposition surrounding mesh fibers in the sham group (Fig 1B).

Figure 1



Conclusions: A 2-dose regimen of PEP injection did not result in bladder mesh exposure resolution for exposures 1x2cm in size. As suggested by the presented modest tissue regeneration and reduction in mesh exposure area, PEP may be efficacious for smaller exposures; further study is warranted.

Research area: female pelvic medicine; regenerative medicine; innovative technologies

Aging and the female urinary microbiome: associations between *Lactobacilli*, menopause, and vaginal estrogen use

Diaz, Nicole[1]; Karstens, Lisa [2]; Wang, Zhuoqun [3]; Ma, Li [3]; Siddiqui, Nazema, Y[1]

[1]Duke University Hospital, Durham, NC; [2]Oregon Health and Science University, Portland, OR
[3]Duke University Department of Statistical science, Durham, NC

Introduction/Objectives: The urinary microbiome demonstrates variations that are linked to aging. While prior studies have shown how the composition of the vaginal microbiome changes after menopause, less in known about the bladder microbiome and menopause. We hypothesized that abundance of *Lactobacilli* within the female urinary bladder mirrors what is seen in the vagina. Specifically, we hypothesized that bladder *Lactobacilli* decrease after menopause and increase after at least 6 weeks of vaginal estrogen.

Methods: In this cross-sectional study, participants provided a catheterized urine sample under a research protocol. We sampled 3 groups of women: premenopausal women ages 30-45 (Pre), postmenopausal women > 55 years of age (Post), and postmenopausal women using vaginal estrogen for at least 6 weeks prior to sampling (Post+vagE2). Samples, negative controls, and standard aliquots of a mock microbial community (positive controls) underwent DNA isolation and Illumina 16S rRNA sequencing. A DADA2 bioinformatic pipeline that allows for identification of taxa at the species level was used. Abundance patterns for several clinically-relevant genera were compared using Kruskal-Wallis with *post hoc* false-discovery-rate (FDR) corrected pairwise Wilcoxon rank sum. Alpha diversity indices were compared using ANOVA. Bacterial compositions at the species level, as estimated by weighted UniFrac distances, were compared between groups using PERMANOVA while adjusting for age, body mass index, diabetes, sexual activity, probiotic use, race/ethnicity, and presence of overactive bladder.

Results: Of the total n = 166 enrolled participants, we recovered sequencing data for n = 150 (31 Pre, 61 Post, 58 Post+vagE2) urine samples. Stacked bar blots illustrate common genera, with significant differences in relative abundances (Figure 1). Comparing alpha diversity metrics by study group showed no differences in total observed species while indices assessing a combination of richness and evenness were significantly different and showing the highest amounts of diversity in postmenopausal women without estrogen (Figure 2). In adjusted PERMANOVA model, microbial compositions (estimated by weighted UniFrac distance) were significantly different between Pre, Post, and Post+vagE2 groups (p=0.012). Increasing age (years) and body mass index also remained associated with microbial composition (p=0.005 and p=0.047, respectively) in the adjusted model.

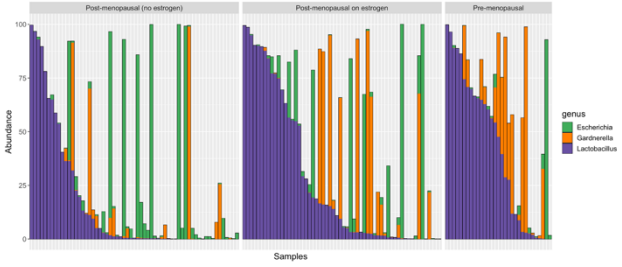


Figure 1: Stacked bar plots illustrating relative abundance of 3 clinically relevant genera (Escherichia, Gardnerella, Lactobacillus). All other genera are shaded in gray. Data generated using V4 amplicon 16S rRNA sequencing, with a DADA2 bioinformatics pipeline. Taxonomy assigned using BLCA with the SILVA reference database.

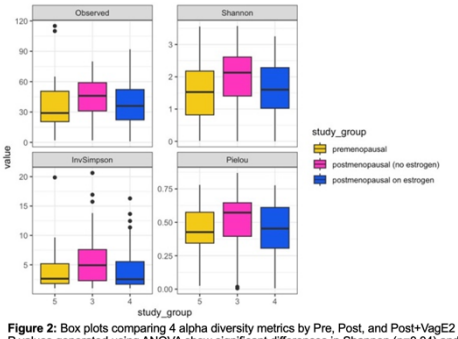


Figure 2: Box plots comparing 4 alpha diversity metrics by Pre, Post, and Post+VagE2 groups. P values generated using ANOVA show significant differences in Shannon (p=0.04) and Pielou (p=0.05) indices by group, but not in observed species (p=0.10) or Inverse Simpson (p=0.12).

Conclusions: Consistent with our hypothesis, the female bladder microbiome has demonstrable changes after menopause, including increased diversity and reduced abundance of *Lactobacilli.* Treatment with at least 6 weeks of vaginal estrogen is associated with an increase in *Lactobacilli* and decreased alpha diversity, to ranges that are more consistent with the premenopausal state.

Research Area: Microbiome

Odd P#s are presented in the AM-session; Even P#s are presented in the PM session

Last Name	First Name	Title	Poster
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Advani	Sonali	Prevalence of and Risk Factors for Bacteremic UTIs in Hospitalized Adults without Definitive Signs or Symptoms of UTI	P-26
Alexander	Molly	Effects of perfluorooctane sulfonate (PFOS) and perfluorooctanic acid (PFOA) exposure on erectile function in Sprague-Dawley rats: A Pilot Study	P-08
Barth	Bradley	Sacral nerve stimulation for constipation in virtual and rodent colons	P-18
Cervantes	Anissa	Resolvin E1 reduces diabetes-associated inflammation but does not improve urodynamic findings of underactive bladder in Akita male mice	P-21
Chen	Junqin	Cavitation Contributes to Energy Delivery and Stone Damage During In Vitro Thulium Fiber Laser Lithotripsy	P-04
Covarrubias	Claudia	The role of atrial natriuretic peptide in the secretion of neurotrophins by bladder smooth muscle cells: an in vitro study	P-15
Diaz	Nicole	Urinary metagenomics (UMETA): A comparison of host cell depletion protocols and sequencing techniques for the urinary microbiome	P-30
Dvergsten	Thomas	Prostate health index density as a means of identifying benign causes of elevated prostate-specific antigen	P-01
Foreman	Jordan	Preoperative bladder mechanics forecast individualized artificial urinary sphincter longevity	P-09
Gonzalez	Eric	AMPLIFY: Amplifying Sensation in Underactive Bladder (Work in Progress)	P-19
Grob	Gabrielle	Functional near infrared spectroscopy (fNIRS) reveals different patterns of neuroexcitation for women with and without overactive bladder during natural filling	P-16
Gultekin	Ata	Specialized pro-resolving mediator Resolvin E1 does not inhibit caspase-1 driven inflammation in murine urothelial cells	P-20
Hajj	Aya	Improvement in Bladder Parameters of 12-Month-Old Male and Female Mice with THX-B Treatment, an Antagonist to the P75NTR Receptor.	P-14
Hudson	Britney	Short- and long-term hypoxic response of bladder urothelial cells in vitro	P-27
Jin	Huixia	Male Akita diabetic mice develop underactive bladder independent of NLRP3 that can be prevented with blood glucose control	P-23
Luchristt	Douglas	Extended treatment-dose antibiotic therapy versus low-dose prophylaxis for the management of recurrent urinary tract infections	P-25
Mazeaud	Charles	Functional activity of the lumbosacral spinal cord by bulbocavernosus reflex stimulation: a pilot functional MRI study	P-12
Merutka	Ilaria	Integrating molecular epidemiology and mechanistic toxicology to unravel Chronic Kidney Disease of Unknown Origin (CKDu)	P-31

Last Name	First Name	Title	Poster
Mishra	Arpit	Thulium Fiber Laser Lithotripsy: Finding the Sweet Spot for Optimal Settings and Maximum Dusting Efficiency	P-02
Mulcrone	Jack	NLRP3 drives bladder denervation during diabetes in the male Akita mice	P-22
Pallas	Wrenn	Characterization of cardiovascular function, metabolic status and erectile function in rat model of cardiometabolic syndrome	P-07
Pope	NiQuava	Diabetes increases cholinergic neurotransmitter-mediated contractions in the type 1 diabetic female Akita mouse due to inflammation mediated by the NLRP3 inflammasome	P-24
Shah	Ashti	Temporally Complex Inflammatory Networks in Animal Model Reveal Signatures for Interstitial Cystitis and Bladder Pain Syndrome Phenotype	P-28
Soto Palou	Francois G	Dusting efficiency for thulium laser fiber: when it comes to frequency, less is more.	P-03
Spellman	Alexandria	Disparities in healthcare utilization and outcomes for older adults with infected stones	P-10
Su	Johnny	Challenges and considerations in delivering botulinum neurotoxin (BoNT) to adults with congenital genitourinary (GU) abnormalities with history of complex GU reconstruction	P-05
Suyama	Julie	Novel magnetic resonance imaging biomarkers of bladder wall composition in a preclinical model of interstitial cystitis/painful bladder syndrome	P-29
Tua-Caraccia	Rafael	Assessing Financial Toxicity in Pediatric Urology: Validation of a Patient-Reported Outcome Measure (PROM) Tool in Spina Bifida	P-11
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Wiener	John	Use of clean intermittent catheterization in children aged two years and under with myelomeningocele: findings from the umpire protocol	P-13

P-01

Prostate health index density as a means of identifying benign causes of elevated prostate-specific antigen

Dvergsten, Thomas G[1]; Dudinec, Johnny[1]; Morris, Kostantinos E[2]; Moul, Judd W[3]

Affiliations: [1]Duke University School of Medicine, Durham, NC; [2]Department of Urology, Indiana University School of Medicine, Indianapolis, IN; [3]Department of Surgery, Division of Urology, Duke University School of Medicine, Durham, NC.

Introduction/Objectives: In the setting of an elevated prostate-specific antigen (PSA), it can be challenging to identify patients in whom this rise is secondary to benign causes, such as benign prostatic hyperplasia (BPH), as opposed to prostate cancer. Much prior work has sought to account for prostate size by considering the PSA density (PSAD) – a measure of PSA per unit volume of gland as assessed on digital rectal exam (DRE), transrectal ultrasound (TRUS), or magnetic resonance imaging (MRI) – as an important screening test, with PSAD less than 0.15 ng/mL·cm^3 generally reassuring for benign processes. Given the development of additional screening tests beyond PSA, particularly the prostate health index (PHI) which stratifies patients into risk categories based on their total PSA, free PSA, and p2PSA, it is important to assess whether these tools can also account for prostate size and correctly identify patients with BPH. It was hypothesized that PHI density – PHI divided by prostate volume – would be able to discriminate between cases of elevated PSA due to benign processes versus cancer and do so in a manner superior to PSAD.

Methods: Patients were selected for this study based on the following criteria: 1) follow-up with the senior author between January and June 2022, 2) prostate biopsy with PHI measurement within 1 year of the procedure, and 3) PSA at time of PHI measurement between 4 and 10 ng/mL. To yield the PHID, the PHI value associated with each biopsy was divided by the prostate size as determined on TRUS at time of the procedure. PSAD was calculated in a similar manner, utilizing the PSA obtained at time of PHI measurement. After categorizing biopsies into 2 groups based on their malignancy status, the associated PSAD and PHID values were compared. Further analysis was performed to compare the ability of PSAD and PHID to differentiate between cases of PSA elevation secondary to benign causes versus cancer. Optimal thresholds for both PSAD and PHID were identified by maximizing Youden's index, and the resulting negative predictive values (NPVs) were assessed.

Results: In sum, 107 patients with a total of 111 biopsies met the criteria and were included in the analysis. Of these biopsies, 41 were without evidence of malignancy, while 70 demonstrated cancer (Gleason 6: n = 39, Gleason 7: n = 24, Gleason 8: n = 3, Gleason 9: n = 4). Mean (±SD) age at biopsy was 63±7 years, and mean prostate volume at biopsy was 54±24 cm^3, with only a single patient having a prostate volume <20 cm^3. Among biopsies without evidence of malignancy, the mean PSAD was 0.13±0.05, whereas that for biopsies with demonstrated cancer was 0.15±0.07 ($p = 0.08$). Mean PHID values for biopsies without and with malignancy were 0.89±0.50 and 1.24±0.85 ($p < 0.01$), respectively. Maximizing Youden's index yielded optimal thresholds of 0.14 for PSAD and 0.79 for PHID. Applying these to differentiate between cases of benign PSA elevation and cancer resulted in NPVs of 42% and 51% for PSAD and PHID, respectively.

Conclusions: In patients with PSA values between 4 and 10, PHID outperforms PSAD in identifying cases of PSA elevation due to benign processes. Work is ongoing to expand the cohort and further explore this finding.

Research area: Benign prostatic hyperplasia

Thulium Fiber Laser Lithotripsy: Finding the Sweet Spot for Optimal Settings and Maximum Dusting Efficiency

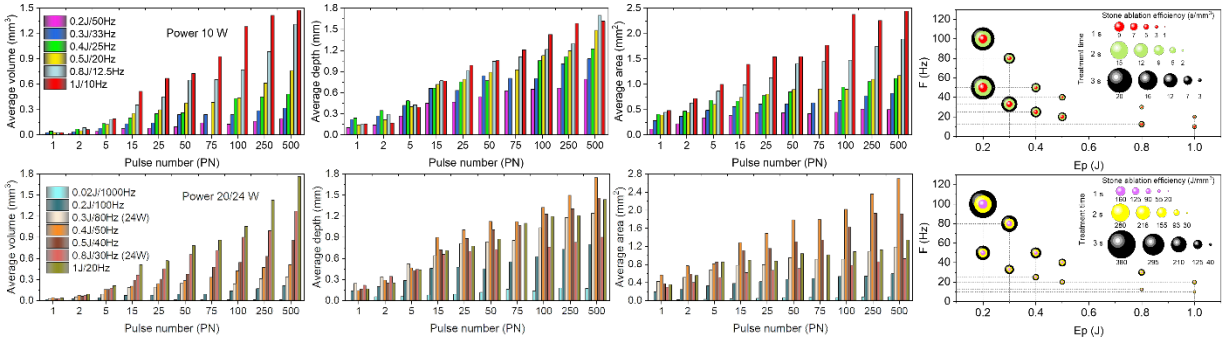
Mishra, Arpit[1]; Chen, Junqin[1]; Zhong, Pei[1]

[1] Thomas Lord Department of Mechanical Engineering and Materials Science, Duke University, Durham, North Carolina

Introduction: Thulium Fiber Laser (TFL) technology is often employed based on empirical experience and conventional dusting settings established from Holmium (Ho): YAG lasers and clinical trials. TFL clinical platforms offer high frequency (up to 2400 Hz) and a broad range of pulse energy from 0.025 J-6 J. Scientific evidence, however, is lacking to substantiate the most efficient settings due to the fundamental differences in pulse patterns and energy delivery mechanisms between TFL and Ho: YAG lasers. This study aims to determine the optimal settings for stone dusting using TFL through examining the treatment outcome produced by a variety of combinations of pulse energy (E_p) and frequency (F).

Methods: To emulate clinical TFL platform settings, an IPG TFL system (TLR-50W, IPG Photonics, Oxford, MA) treated a 23 x 23 x 4 mm BegoStone sample (5:2 water-to-powder ratio, BEGO USA, Lincoln, RI). Stones were soaked for 24 hours before treatment. A 200 µm fiber (HHz Connector™ Laser Fiber, NA = 0.22, AZ) perpendicular to the stone surface submerged in a water tank delivered 1 to 500 pulses (n = 5) at various E_p, and F combinations for 10-24 W power. Each E_p/F combination was examined at a standoff distance (SD) of 0.5 mm, i.e., in clinically recommended non-contact mode (fiber-to-stone distance ≤ 1 mm). Following the treatment, characterization of the resultant craters (crater volume, average depth, and profile area) was performed using the optical coherence tomography (OCT). Subsequently, we established the optimal settings for stone dusting at SD = 0.5 mm in terms of treatment time (s/mm^3) and energy delivered (J/mm^3).

Results: The crater volume increases in general with the pulse number delivered and the laser power. While there was no discernible trend in depth and profile area at 10 W, they culminated at 0.4 J/50 Hz at 20 W. This indicates that traditional photothermal ablation is not the only mechanism for stone removal with TFL lithotripsy, and that the dynamics of cavitation bubbles may also be important. The optimal settings for efficient stone dusting with the TFL were observed to be at high E_p and low F. TFL achieved maximum dusting efficiency with the shortest treatment time (s/mm^3) and lowest energy (J/mm^3) required per unit ablated volume at 1 J/20 Hz and 1 J/10 Hz, respectively.



Conclusion(s): These findings provide insight into the most effective TFL settings. However, further studies are needed to comprehend the underlying mechanism responsible for these optimal settings and to assess their feasibility using human stones in an artificial kidney model under more clinically relevant treatment conditions.

Research area: Nephrolithiasis

Dusting efficiency for thulium laser fiber: when it comes to frequency, less is more.

Soto Palou, Francois G.; Chen, Junqin; Medairos, Robert; Antonelli, Jodi; Zhong, Pei; Preminger, Glenn; Lipkin, Michael

Duke University Medical Center, Department of Urology

Introduction & Objective:
Data from Holmium laser studies has taught us that optimal dusting generally require low energy and high frequency settings. In trialing novel TFL technology urologists have often relied on “traditional” dusting settings obtained from Holmium laser trials and experiments. However, these two platforms are fundamentally different in their pulse profile and energy delivery. Clinical TFL platforms have an energy range of 0.025J-6J and a frequency range of 1Hz-2400Hz. With the seemingly endless combination of settings and lack of scientific evidence to support one over the other we aim to assess the efficiency of the TFL platform in an automated *in vitro* “dusting model”.

Methods:
All tests were conducted using an IPG Photonics TLR-50W TFL system and a 200μm fiber on “soft” (5:2) Begostone phantoms. We selected the most popular dusting settings (Figure 1) among endourologist familiar with TFL[1] and tested each setting at four different standoff distances (SD) (0.2mm, 0.5mm 1mm, 2mm) and at clinically significant scanning speeds of 1mm/sec or 2mm/sec. Our previous benchtop studies have shown that short pulse provides superior ablation, so all pulse profiles were adjusted to maximum peak power and short pulse. The laser fiber was scanned in a 15mm straight line with a 3D positioning system across a polished and submerged Begostone surface. Ablation volumes of 4mm and 2mm trough segments were quantified by optical coherence tomography (OCT).

Results:
Greatest stone ablation was achieved at high energy and low frequency settings ($p<0.005$). Overall, the settings that produced the greatest ablation volume were 1J/10Hz (2.51mm^3) and 1J/20Hz (2.55mm^3). Predictably, as SD increased ablation volume decreased.

Conclusions:
The most efficient dusting settings while dusting with the TFL occur at high energy, low frequency, and at a SD of 0.2mm. Further studies are required to quantify size of dust particles produced by ablation at these settings.

10 W (v_{fiber} = 1 mm/s) vs. 20 W (v_{fiber} = 2 mm/s) (40 J)

Power (W)	E_p (J) X F (Hz)	Fixed Peak Power (100%)	
		Pulse Duration (ms)	% Peak Power
10 (4 mm troughs)	0.05 x 200	0.20	45
	0.10 x 100	0.20	90
	0.20 x 50	0.35	100
	0.40 x 25	0.70	100
	0.50 x 20	0.87	100
	1.00 x 10	1.73	100

Power (W)	E_p (J) X F (Hz)	Fixed Peak Power (100%)	
		Pulse Duration (ms)	% Peak Power
20 (4 mm troughs)	0.05 x 400	0.20	45
	0.10 x 200	0.20	90
	0.20 x 100	0.35	100
	0.40 x 50	0.70	100
	0.50 x 40	0.87	100
	1.00 x 20	1.73	100

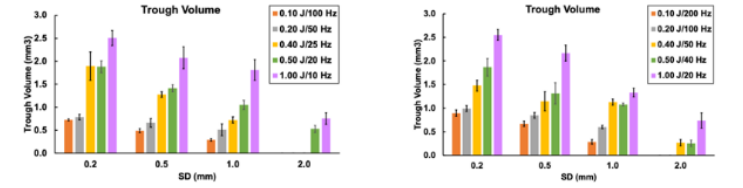


Figure 1:

[1] Sierra A, Corrales M, Piñero A, Traxer O. Thulium fiber laser pre-settings during ureterorenoscopy: Twitter's experts' recommendations. *World J Urol*. 2022;40(6):1529-1535. doi:10.1007/s00345-022-03966-9

Research area: Nephrolithiasis, Innovative technologies

P-04

Cavitation Contributes to Energy Delivery and Stone Damage During *In Vitro* Thulium Fiber Laser Lithotripsy

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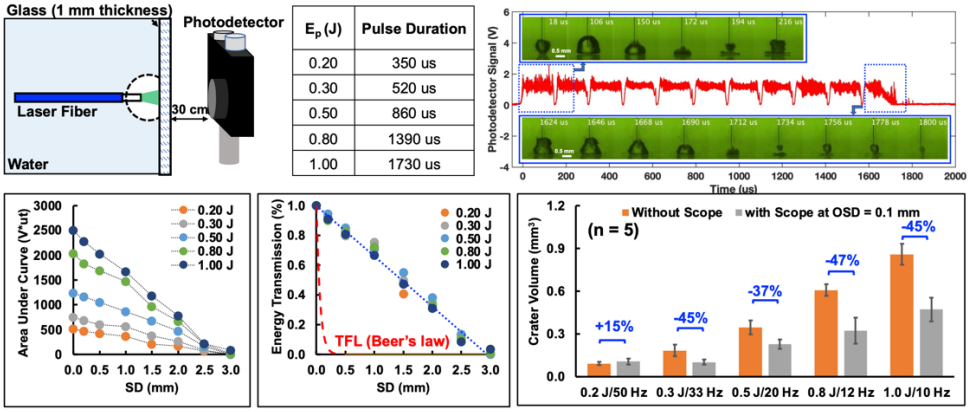
[1] Thomas Lord Department of Mechanical Engineering and Materials Science, Duke University, Durham, North Carolina

Introduction: Thulium fiber laser (TFL) lithotripsy is gaining popularity due to its high efficiency in treating urinary tract stones. However, few mechanistic studies have been carried out to understand the laser-bubble-stone interaction produced by TFL, impeding the optimal use of this new lithotripsy technology. In this study, we aim to investigate the role of cavitation in energy delivery and stone damage produced by TFL under various laser energy (E_p) settings.

Methods: Experiments were conducted using a TFL system (TLR-50W, IPG Photonics) with 200 μm fiber to generate rectangular-shaped laser pulses with varying E_p levels ranging from 0.2 to 1.0 J (see Figure below). To investigate the correlation between bubble activities and laser energy delivery, the fiber was immersed in water and placed perpendicular to a 1 mm-thick fused quartz slide. The standoff distance (SD) between the fiber tip and slide varied from 0 to 3 mm. An InGaAs photodetector (PDA10D, Thorlabs, Newton, NJ) was placed 30 cm away in the air on the opposite side of the slide to examine the temporal pulse power profile of the transmitted light. The concomitant dynamics of the bubble were captured using high-speed imaging. Furthermore, BegoStone samples were treated in water with 50 pulses delivered at SD = 0.5 mm. We performed the experiments with and without a ureteroscope, and in the latter case, we offset the scope distance by 0.1 mm to alter the dynamics of bubble collapse and assess its contribution to stone damage.

Results: The laser energy can transmit through the vapor tunnel the bubble creates during its expansion phase (i.e., Moses effect) and suspend during the bubble collapse. After integrating the power profiles over time, we have found that greater energy transmission can be achieved at higher E_p and smaller SD. Interestingly, at a fixed SD, the percent of incident laser energy that can transmit through the slide, i.e., reach to the stone surface remains almost constant for all E_p's. Furthermore, when the bubble was distracted away from the stone surface by the ureteroscope tip, stone damage was significantly reduced by 37%-45% compared to the treatment without the scope, except for E_p = 0.2 J.



Conclusion: This study demonstrates the importance of cavitation in energy delivery to the stone via the "Moses effect". The bubble collapse with jet impact during TFL lithotripsy may contribute either directly to stone damage or indirectly to dust removal from the crater surface and thus enhancing photothermal ablation effect, which needs to be further investigated.

Research Area: Nephrolithiasis

P-05

Challenges and considerations in delivering botulinum neurotoxin (BoNT) to adults with congenital genitourinary (GU) abnormalities with history of complex GU reconstruction

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Introduction: While intradetrusor BoNT injection is a well-established treatment of neurogenic detrusor overactivity, its administration in patients with GU abnormalities or complex reconstruction can be difficult. The objective of this report is to highlight our institution's experience with challenges and solutions in delivering BoNT to this unique patient population.

Methods: We performed a retrospective review of our institution's database of adult patients with congenital GU abnormalities with complex reconstruction who received intradetrusor BoNT injections performed by a single surgeon between 2016 and 2023. In patients with multiple BoNT injections, the most recent procedure was used as the index case for analysis.

Results: A total of 16 patients were included, with a mean age of 28 years (range 21 - 46). The most common diagnosis was myelomeningocele (n=12), followed by sacral agenesis (n=2), Williams syndrome (n=1), and urethral atresia (n=1). The most common GU reconstruction was augmentation cystoplasty (n=11). 75% (12/16) of the patients had a continent catheterizable channel (CCC) and 31% (5/16) had undergone prior bladder neck reconstruction or closure.

Challenge	Suggestions or Solutions
High riding bladder neck	• Making sure the patient is at the end of the table with no trays obstructing lowering of the scope • Using a flexible cystoscope
Reconstructed bladder neck (e.g. Leadbetter, fascia sling/wrap)	• Using a mini nephroscope • Pediatric cystoscope can be considered in some women *(but it still does not reach posterior and lateral wall adequately)*
Urethral stricture disease due to frequent CIC	• Using a mini nephroscope *(pediatric cystoscope will not reach the bladder for men and most women)*
Presence of AUS	• Using a mini nephroscope • Avoid injection around the trigone and dome to avoid injury to the cuff, balloon, or tubing
Closed bladder neck	• Consider accessing the bladder via CCC or suprapubic tract with a flexible cystoscope
Small caliber continent stomas	• Not widely available: small diameter or pediatric flexible cystoscope
Stomal stenosis at the skin level	• If indicated, perform stoma revision or balloon dilation and then proceed with delivering BoNT
Presence of augmentation cystoplasty	• Avoid injection close to the anastomotic line • Prefer injecting into the native detrusor but also safe/effective to inject into the bowel segment if surface area is limited

Conclusions: Additional considerations and instruments are required for safe and effective BoNT injections in patients with congenital GU abnormalities and reconstruction. Adequate preparation may be needed (e.g. performing injections in the operating room under anesthesia).

Research area: Neurourology, Urinary Reconstruction

P-06

Current evidence-based strategies for management of penile prosthesis infections

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[1]Department of Surgery, Division of Urology, Duke University Medical Center, Durham, North Carolina.

Introduction: Implantation of a penile prostheses is a durable definitive treatment for men with erectile dysfunction (ED) refractory to less invasive treatment modalities. The frequency of penile prosthesis implantation has decreased steadily since the mid 2000's due to technological improvements in penile prosthesis technology such as antibacterial coating and improvements in surgical techniques such as the "no-touch" technique, but penile prosthesis infections still occur and is a devastating complication. We review the evidence for strategies to manage a patient with a penile prosthesis infection.

Methods: We conducted a narrative review of the literature on management of penile prosthesis infections. Publications were identified by searching electronic databases and included current guidelines, case series, case reports, previous reviews, and expert opinions. Only peer-review publications available in English were considered for this review.

Results: Historically, patients who presented with penile prosthesis infections were exclusively managed with complete removal of the prosthesis without replacement. Although this strategy is safe it precipitates immediate severe ED and leads to corporal fibrosis and penile length shortening. The "Mulcahey Salvage" entails removal of an infected prosthesis and washout followed by immediate inflatable penile prosthesis (IPP) replacement was popularized after a landmark paper in 1996 showing this technique is safe with a low reinfection rate. A delayed salvage protocol did not have a lower reinfection rate, so the Mulcahey Salvage became the standard for patients able to undergo salvage surgery. Advancements to this technique include using antiseptic irrigation solutions during surgery, antibiotic protocols based on intraoperative culture data from infected penile prostheses, and the use of malleable prosthesis have subsequently been shown to have an improved reinfection free success rate. A few recent small case series have reported successful treatment of localized penile prosthesis infections with conservative treatment without penile prosthesis removal, but the standard of care for management of a penile prosthesis infection continues to include removal of all prosthesis device components.

Conclusion: The "Mulcahey Salvage" and "Malleable Implant Substitution Technique" are the optimal strategies for management of most patients who present with a penile prosthesis infection. These strategies prevent the development of corporal fibrosis from prosthesis removal without replacement and have clinical evidence of safety and efficacy with low reinfection rates.

Management Strategy	Advantages	Disadvantages
• Penile prosthesis removal without immediate replacement (With or without prosthesis reimplantation in ~3 months)	• Lowest risk of continued infection because no hardware remains in place. • Shorter surgical time than removal with immediate replacement.	• Does not allow for continued treatment of patient's ED without a second surgery to replace the prosthesis. • Allows for corporal fibrosis which leads to decreased penile length and more difficult subsequent reimplantation.
• Mulcahey Salvage = Penile prosthesis removal with immediate replacement with an IPP	• Allows patient to have continued treatment of ED with an IPP in a single operation. • Prevents corporal fibrosis & loss of penile length.	• Most extensive immediate surgical operation requiring replacement of entire IPP device.
• "Malleable Implant Substitution Technique" (MIST) = Penile prosthesis removal with immediate replacement with a malleable penile prosthesis (With or without interval surgery to replace malleable prosthesis with an IPP)	• Allows patient to use malleable prosthesis for ED as possible definitive treatment, or until replaced by IPP. • Prevents corporal fibrosis & loss of penile length. • Does not require replacement of scrotal pump, reservoir, or tubing.	• Lower patient satisfaction with malleable prosthesis than IPP. • Patient would require a second surgery in the future if they desire replacement with an IPP.
• Antibiotic treatment without penile prosthesis removal (With or without excision and drainage)	• Less extensive surgery than penile prosthesis removal and replacement. • Allows continued treatment of patient's ED • Does not cause corporal fibrosis & loss of penile length. • If unsuccessful, other treatment options are still available.	• Limited evidence for successful utilization in clinical literature. • Many patients may not be good candidates for this strategy. • If conservative treatment is unsuccessful, the patient will ultimately still require surgery prolonging time to successful recovery.

Research Area: Sexual Medicine, Infection Management, Urologic Medical Devices

P-07

Characterization of cardiovascular function, metabolic status and erectile function in rat model of cardiometabolic syndrome

Authors: Pallas[1*], Wrenn D., Hannan[1], Johanna L.

Affiliations: Brody School of Medicine, East Carolina University, Physiology Department, Greenville, North Carolina, USA[1]

Introduction/Objectives: Cardiometabolic syndrome is a widespread health issue and common cause of erectile dysfunction (ED), yet the molecular basis of ED in cardiometabolic syndrome needs to be elucidated. Our aim is to characterize the cardiovascular and erectile function, and metabolic status in the obese ZSF-1 rat. This hybrid rat is a cross between a Zucker diabetic fatty female and a spontaneously hypertensive heart failure male rat.
Methods: We used male lean and obese ZSF-1 rats (n=8/group). We measured body weight weekly, and performed MRI and glucose tolerance tests at 10, 20, 30 weeks. At 30 weeks, we assessed erectile function using ICP/MAP and vascular stiffness was measured via laser doppler pulse wave velocity (PWV). Additionally, tissue bath experiments measured acetylcholine (Ach)-mediated vasodilation, vasodilation to sodium nitroprusside (SNP), and contraction to phenylephrine (PE) in thoracic and abdominal aortas. Aorta and penile segments were collected for histological analyses.
Results: On average, obese rats were 60% larger (230g) than controls and had significantly impaired glucose tolerance at all time points ($p<0.05$). At 30 weeks, obese ZSF-1 rats had significantly reduced ICP/MAP compared to controls indicating the development of erectile dysfunction ($p<0.05$). Obese rats were significantly more hypertensive compared to lean rats (MAP: 140.2 mmHg vs 99.6mmHg, $p<0.05$). Lean and obese rats had no difference in vascular stiffness indicated by PWV. In our tissue bath experiments, ACh-mediated vasodilation was significantly decreased in the thoracic aorta of obese rats ($p<0.05$) while the abdominal aorta was unchanged. SNP-mediated vasodilation was similar between groups in both thoracic and abdominal aortas. At high concentrations, PE-mediated vasoconstriction was significantly increased in the thoracic and abdominal aortas of obese rats ($p<0.05$).
Conclusions: To our knowledge, this is the first report on the erectile function in the ZSF-1 rat model of cardiometabolic syndrome. We believe that the ZSF-1 rat is useful for studying erectile dysfunction as they gradually develop metabolic disease and erectile dysfunction over time similar to the human disease. Further characterization of the penile smooth muscle physiology, and penile and aortic histology will follow.

Research area: Sexual dysfunction

P-08

Effects of perfluorooctane sulfonate (PFOS) and perfluorooctanic acid (PFOA) exposure on erectile function in Sprague-Dawley rats: A Pilot Study

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[1]Department of Physiology, Brody School of Medicine at East Carolina University, Greenville, NC; [2]Department of Pharmacology and Toxicology, Brody School of Medicine at East Carolina University, Greenville, NC

Introduction: Per and polyfluoroalkyl substances (PFAS) are used in a variety of industrial and consumer products and have been deemed environmental and health contaminants. The most common PFAS chemicals, perfluorooctane sulfonate (PFOS) and perfluorooctanic acid (PFOA), are used in firefighting foam, nonstick bakeware, and fire resistant materials. The impact of PFAS exposure on erectile physiology is unknown. Thus, we will assess therefore the aim of this pilot study is to determine the effect of PFOS and PFOA on erectile function. We hypothesize that PFOS and PFOA will cause detrimental effects and decrease erectile function.
Methods: Adult male Sprague-Dawley rats were divided into three groups (n=4/group): 1) Control; 2) PFOS (10mg/kg/day); and 3) PFOA (10mg/kg/day). PFOS/PFOA were dissolved in 0.05% Tween 20 and administered in drinking water for 28 days. Controls received 0.05% Tween 20 in drinking water. Following 28 day PFOS/PFOA exposures, rats were placed on regular drinking water for 2 weeks. Body weights were collected weekly and terminal organ weights of liver, testes, spleen, heart, lungs, brain, seminal vesicles, kidneys, bladder, and thymus were recorded and normalized to body weight. Prior to PFAS exposure and every 2 weeks thereafter, apomorphine-induced behavioral erections and yawns were measured.
Results: Body weight was significantly decreased in PFOS rats at 28, 35 and 42 days (D28: 414.3g; D35: 432.2g; D42: 445.4g) compared to controls (D28: 509.0g; D35: 528.1g; D42: 543.8g). However, PFOA exposure did not change body weight. Both liver and testes weights were significantly higher in PFOS treated rats compared to PFOA and control rats ($p<0.0001$). Additionally, PFOS treated rats had significantly lower spleen to body weight ratios compared to control and PFOA treated rats ($p<0.001$). No other organ weights were different across exposure groups. Apomorphine-induced erections in PFOS treated rats were lower at 2 weeks and completely absent at 4 and 6 weeks from initiation of exposure ($p<0.001$). PFOA exposed and control rats had similar numbers of erections throughout the 6 week period. The number of yawns were also markedly decreased in PFOS rats at 4 and 6 weeks. Erectile responses will be confirmed with direct measurement of nerve-stimulated intracavernosal pressure and mean arterial pressure.
Conclusions: PFOS exposure had marked decrease on body weight, increased liver and testes size, and decreased spleen weights. Erectile function was severely decreased after 4 weeks of PFOS exposure. PFOA exposure did not cause any systemic organ changes or impact erectile function. Ongoing studies will repeat these exposure parameters in more animals to confirm our results and whether PFOS is severely impacting erectile function.

Research Area: Sexual Function, Toxicology/Environmental Health, and Erectily Function

P-09

Preoperative bladder mechanics forecast individualized artificial urinary sphincter longevity

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Introduction/Objective: Time to artificial urinary sphincter (AUS) removal/replacement is variable with no current means of forecasting individualized survival times. We developed a prediction instrument for time to AUS removal/replacement.

Methods: Post-prostatectomy patients undergoing first-time AUS placement from 2000 to 2021 were identified excluding causes related to erosion and infection. AUS survival, defined as time to replacement, was tested against clinical, urodynamic and surgical attributes. Variables were considered for Cox-proportional hazard modeling based on theoretical associations with increased device use and exposure to pressure fluctuations in situ. Excluded records served as a pseudo-validation cohort.

Results: 341 records with urodynamic data were identified. A training cohort of 97 was derived with complete data and median follow-up of 4.2 years (1.7, 8.3). There were 20 AUS replacements (20.6%), mean 5.9 +/- 2.5 years for atrophy (55.0%) and mechanical failure (45.0%). A model was specified on five predictors: bladder neck contracture (BNC) (HR=10.9, p<0.01), detrusor overactivity (DO) (HR=7.3, p<0.01), 24-hour pad weight (HR=0.99, p=0.09), bladder volume which generates a strong sensation to void (HR=0.99 p=0.04) and baseline abdominal pressure (HR=0.92, p=0.07). The c-index was 0.82 for the training cohort and 0.92 for the pseudo-validation cohort of 38 records with 5 (13.2%) AUS revisions. The baseline survival function without risk factors is compared in Figure 1 to that with DO, BNC, and lower quartile of continuous predictors in additive fashion.

Conclusion: Bladder mechanics and more frequent device use appear to be robust predictors of long-term AUS survival. A five-item prediction model demonstrated good discriminative capacity for time to AUS removal/replacement.

Research Area: Urinary Reconstruction, Data Science / Predictive Analytics, Urodynamics

P-10

Disparities in healthcare utilization and outcomes for older adults with infected stones

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Introduction: An obstructing urinary tract stone complicated by infection (septic stone) requires urgent intervention with both antibiotics and urinary tract decompression. Delayed treatment can lead to significant morbidity and sometimes mortality. Given a rapidly aging population and advances in critical care, we sought to examine healthcare utilization and outcomes in patients with sepsis secondary to obstructing stones in a contemporary cohort, with a focus on older adults.
Methods: We conducted a retrospective cohort study using data from the nationwide Premier Healthcare claims database (2016-2020) to identify hospital inpatients aged 18-95 treated for septic stones, using ICD-10 diagnostic codes consistent with sepsis and urinary stones. Patients were stratified into two age groups: adults $\geq$ 65 years old ("older adults"), and adults aged <65 years (the "younger cohort"). Multivariable regression models were adjusted for sociodemographic factors and comorbid conditions. Hospital mortality, discharge status, and 30-day readmission were compared in the two groups.
Results: The study cohort included 27,976 patients of whom 54.7% were older adults. The overall cohort had an average age of 64.1 years (SD = 16.2). 41.7% were male and 76.6% were non-Hispanic white individuals. In unadjusted analyses, older adults received higher levels of care (invasive mechanical ventilation, vasopressor use), had a longer length of stay, incurred higher costs, and had a higher mortality. In adjusted analyses, older adults had a higher mortality (OR 2.14, 95% CI 1.84-2.49, p<0.0001) and were less likely to be discharged home (OR 0.42, 95% CI 0.39-0.45, p<0.0001).
Conclusions: Our data highlight that older adults encounter a disproportionately higher acuity and burden of septic stone disease. Healthcare utilization for septic stones is higher for older adults across multiple dimensions including hospital costs, length of stay, and post-discharge care. Additional investigation is needed to identify patient, clinician, and facility-level drivers of these outcomes to create pathways to reduce disparities and optimize outcomes for older adults.

Research Area: Health Services Research, Nephrolithiasis, Infections of the Urinary Tract

Assessing Financial Toxicity in Pediatric Urology: Validation of a Patient-Reported Outcome Measure (PROM) Tool in Spina Bifida

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Introduction: There is significant recent attention by the public and policy makers in identifying factors that influence healthcare spending to better understand how such costs impact patients, families, and communities.[4] The concept of "financial toxicity" is the negative therapeutic side effect of healthcare-related expenditure on individuals undergoing medical treatment.[5-7] Financial toxicity differs from out-of-pocket cost in that it quantifies subjective distress from expenditure rather than absolute expenditure itself. Interest exists in evaluating financial toxicity as a patient-reported outcome measure (PROM) to quantify cost-related strain.[11] Little is known regarding the impact of financial toxicity on patients with congenital conditions, including those of urological interest, such as spina bifida.[14] Urologic complications and their management, are major sources of morbidity and healthcare-related expense for spina bifida patients and families.[17-23] A better understanding of spina bifida-related financial toxicity (SBFT), including, tools for clinicians to readily identify patients at risk for financial toxicity, are needed. Through the conduction of semi-structured patient/caregiver cognitive interviews (Pro00107310) the study team identified themes related to the financial burden experienced by patients with spina bifida and their caregiver. These themes from the semi-structured qualitative interviews, expert opinion, literature search and previously published and validated COST-FACIT by DeSouza et al[12,13] were used to develop spina bifida specific financial toxicity PROM assessment instruments for spina bifida patients and their caretakers. The aim of this study is to validate and employ the SBFT-PROM tool.

Methods: Patients with Spina Bifida completed a survey for assessing financial toxicity consisting of 19 questions, and parents of patients with Spina Bifida completed the parent version of the survey with 20 questions. For each version of the survey, we conducted descriptive analyses of survey questions to check data quality, confirmatory factor analysis to examine the factor structure, and assessed the reliability of the surveys.

Results: Of the 180 patients, 67% had a household income of less than $50,000. Of the 93 parents, 30% had a household income of less than $50,000. The results of confirmatory factor analyses found that a one-factor model fit the patient survey data well, with a CFI=.96, RSMEA=.08, and SRMR=.05. A one-factor model represents the parent survey data well with a CFI= .93, RSMEA=.12, and SRMR=.09 The factor structures were shown to be reliable with an internal consistency of .94 for the patient survey and .94 for the parent survey.

Conclusion: This marks a significant step in the development of a validated financial toxicity PROM tool that may be used to assist with the identification of Spina Bifida patients and caregivers who are at risk of financial toxicity. The use of reliable and internally consistent survey questions may serve to guide clinicians in their counseling and identification of patients who might benefit from early case management/social work intervention.

Research Area: Pediatric Urology, Clinical Outcomes Research, Health Sciences Research

Functional activity of the lumbosacral spinal cord by bulbocavernosus reflex stimulation: a pilot functional MRI study

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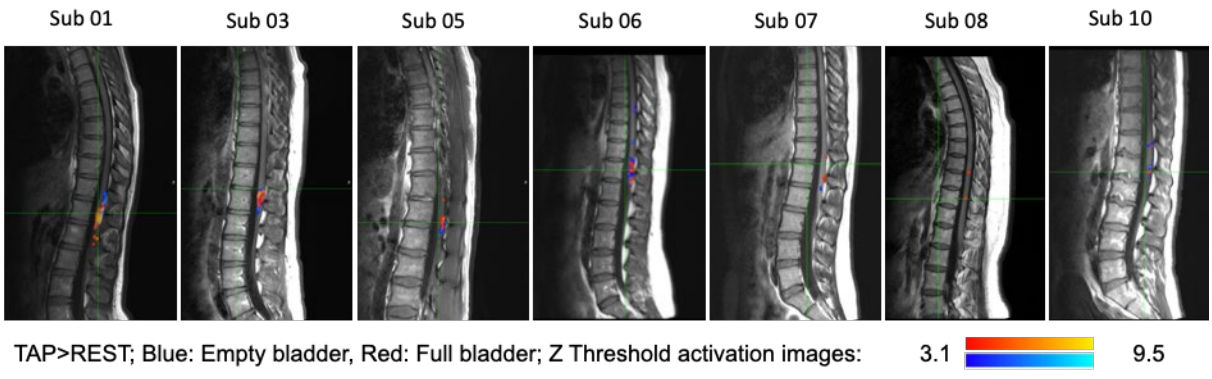
Introduction: The lumbosacral spinal cord is a critical component of the central nervous system, involved in the control of bladder function. Performing functional MRI (fMRI) to analyze neural activity in this region is challenging due to its size, local anatomy, and motion artifacts. Despite these challenges, exploring the lumbosacral neural circuits is essential since it represents the level of control closest to the innervated pelvic organs (bladder, sphincters). Additionally, the bulbocavernosus reflex (BCR), a reliable clinical test, can be used to assess the integrity of the S2-S4 levels of the spinal cord. Therefore, our study aimed to evaluate the feasibility of determining spinal cord activity triggered by BCR utilizing fMRI in healthy individuals.
Methods: We included healthy males and females without any history of neurological disease or urological symptoms. Participants were asked to consume 500ml of water and empty their bladder before entering the MRI room. To elicit the BCR, we developed an MRI-compatible device to perform mechanical suprapubic tapping over the bladder. First, we acquired a 3T MRI sagittal anatomical sequence, followed by a task block fMRI protocol defined by: 20s of tapping and 20s of rest, repeated four times at empty and full bladder states. Data were processed in the subject space (denoising and co-registration to anatomical) with the Spinal Cord Toolbox after manual masking of the spinal cord area with FSL; then, statistical analysis was performed with FSL. A 'Z' score thresholded >3.1 was used with a p>0.05.
Results: Ten individuals (6 females and 4 males), with an average age of 27.6 years, participated in the study. One subject was removed from the analysis due to technical issues during the fMRI scan. A significant signal was detected for empty and full bladder tapping in 7 subjects (Figure 1). Two individuals showed no activation during the two-bladder states. Activated areas observed covered the terminal region of the spinal cord with sparse rostral activity.
Conclusion: We demonstrated here for the first time that noninvasive BCR stimulation can generate significant activation of the lumbosacral spinal cord, visible with fMRI, in healthy individuals. Our results open the door to other analyses of this anatomical region with dedicated stimulation of pelvic organs such as the lower urinary tract.
Research Area: Uroradiology, Neurourology, Data science

Figure 1: Sagittal view of functional activity while tapping at the empty and full bladder, Sub: subjects



Use of clean intermittent catheterization in children aged two years and under with myelomeningocele: findings from the umpire protocol

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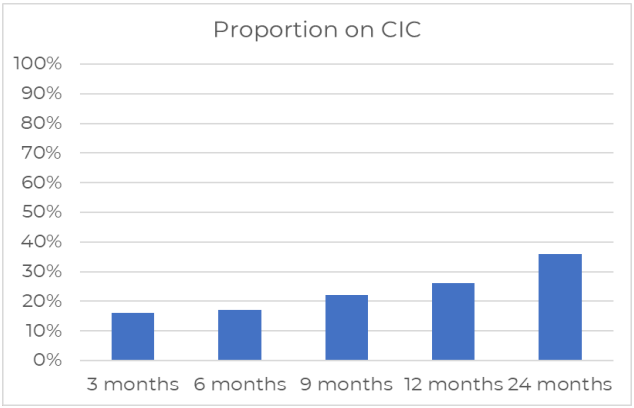
Introduction: Clean intermittent catheterization (CIC) is commonly used in bladder management for individuals with myelomeningocele (MMC). Optimal use of CIC in infants and young children has not been established. We examined CIC use in children aged 2 years and under enrolled in the Urologic Management to Preserve Initial Renal Function Protocol for Young Children with Spina Bifida (UMPIRE) protocol.

Methods: The UMPIRE protocol requires initiation of CIC among all newborns with MMC soon after birth and weaning if volumes remain low. Clinical indicators for resuming CIC in the protocol are: hydronephrosis, high-risk bladder, grade 5 vesicoureteral reflux, or clinical discretion. We evaluated hydronephrosis, urodynamics, and vesicoureteral reflux, and anticholinergic use at routine time points until 2 years of age (0, 6, 12, 18 and 24 months). We compared clinical and sociodemographic characteristics between the baseline and 2-year visit using Chi-square tests of independence.

Results: 232 participants were included and 17% required CIC at 3 months of age. The proportion requiring CIC increased to 26% at 12-15 months and 35% at 2 years of age. CIC use at 2 years was not associated with sex, race/ethnicity, insurance, prenatal closure, level of lesion, shunt, or hydronephrosis, but was associated with presence of vesicoureteral reflux (p=0.02) and anticholinergic use (p<0.001).

Conclusions: Following our strict protocol, less than one-fifth of newborns with MMC required CIC, but that proportion doubled within the first two years of life. Because bladder characteristics can change over time, findings indicate that routine surveillance to monitor clinical changes may help inform re-initiation of CIC.



Research Area: Pediatric Urology, Neurourology, Clinical Outcomes Research

P-14

Improvement in Bladder Parameters of 12-Month-Old Male and Female Mice with THX-B Treatment, an Antagonist to the P75^{NTR}Receptor.

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Introduction: Neuron development and survival are regulated by neurotrophins, including Nerve Growth Factor (NGF). In female elderly people with overactive bladder syndrome (OAB), the levels of NGF in urine are reduced, which has been linked to elevated activity of the enzyme matrix metalloproteinase-9 (MMP-9), the main protease responsible for NGF degradation. These characteristics were also observed in animal models of type 1 diabetic bladder dysfunction, and improved by chronic treatment with THX-B, an antagonist of the proinflammatory receptor p75^{NTR}. The aim of the present study is to investigate the functional benefit of p75^{NTR} antagonism on bladder function in aging mice.

Methods: Male and female C57BL/6J mice, aged 6 and 12 months, were subjected to a four-week treatment of THX-B or PBS (control). Urination behaviors and patterns were measured using a voiding spot test. Bladder contractility was evaluated using organ baths in the presence of KCl, Electrical Field Stimulation, and Carbachol. Conscious cystometry was performed after bladder catheterization surgery to assess bladder contractility. ELISA kits were used to measure NGF and proNGF levels in urine. Immunoblotting was used to semi-quantify MMP-9, VachT, and PGP 9.5.

Results: THX-B improved voiding behavior and bladder contractility compared to the control group: total urine volume, volume per micturition and voiding frequency were reduced in 12-month-old male and female mice. Ex Vivo, bladder contractility stimulated by KCl, Electrical Field Stimulation and stimulation by Carbachol were reduced in strips from mice after THX-B treatment. In female mice, conscious cystometry revealed a decrease in maximal voiding pressure, basal pressure, spontaneous activity, and micturition volume by THX-B treatment compared to controls. In males, THX-B decreased the maximal voiding pressure and residual volume. Moreover, THX-B treatment increased NGF urine levels in 6 and 12-month-old male and female mice, restoring the NGF/proNGF imbalance. MMP-9 activity was decreased, but only in female mice.

Conclusion: Our results suggest that the improved bladder parameters by THX-B is age-dependent and leads to improved bladder function only in 12-month-old mice. Decreased MMP-9 activity occurs exclusively in female mice, suggesting gender specific pathways. THX-B could potentially be used as a therapeutic intervention to improve OAB, in female aging population.

Research Area: Neurourology, Overactive Bladder (OAB), Voiding Dysfunction/Urinary Retention

The role of atrial natriuretic peptide in the secretion of neurotrophins by bladder smooth muscle cells: an in vitro study

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Introduction: Urine storage and voiding by the bladder are controlled by the peripheral and central nervous systems. Neurotrophins are essential for the maintenance and activity of nerve endings. Among them, nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) controls neuroregeneration while their respective precursor proNGF and proBDNF trigger inflammation and apoptosis. A dysregulation in the ratio of neurotrophins to proneurotrophins have been found to be viable biomarkers for overactive bladder syndrome (OAB). On the other hand, our previous data suggests that cGMP plays an important role in neurotrophin secretion and that natriuretic peptide A (ANP), a major source of cGMP, might be involved. The objective of this study was to examine the relationship between ANP and neurotrophins in clinical samples and in bladder cells in vitro. Urine samples from 20 controls and 20 OAB patients (50-80 years) were obtained with validated medical questionnaires.
Methods: ProBDNF and BDNF were measured using specific ELISA kits (Biosensis). Activity of MMP-9 was measured using an enzymatic kit. Smooth muscle cells (SMC) were grown from rat bladder and incubated for 24 hours with ANP (100 nM). ANP, NGF, proNGF, BDNF, proBDNF and matrix metalloproteinase-9 (MMP-9) were measured using specific ELISA kits.
Results: To understand the underlying mechanisms of these results, SMC cultures were treated with ANP (100 nM), which regulates intracellular cyclic GMP (cGMP) levels, a central pathway in the synthesis and secretion of neurotrophins. Our findings indicate that ANP reduces the secretion of NGF and proBDNF by SMCs, while proNGF and BDNF remain unaffected, similar to what was observed in patients with OAB. Additionally, ANP increased the activity of MMP-9 in the same cell culture medium (Figure 1). finally, we found that ANP content in the urine of OAB patients was elevated, supporting our hypothesis (Figure 2).

Figures:

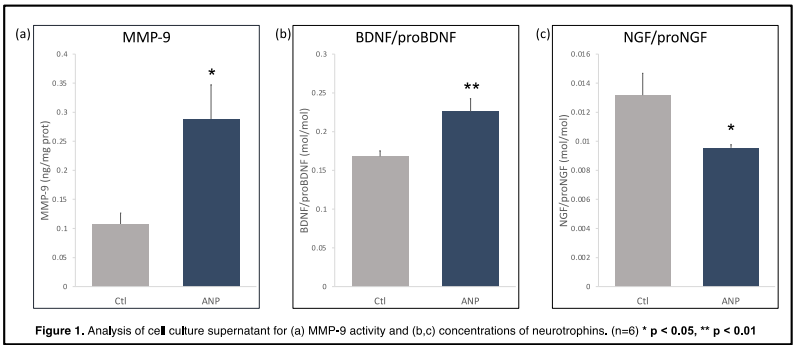

Figure 1. Analysis of cell culture supernatant for (a) MMP-9 activity and (b,c) concentrations of neurotrophins. (n=6) * $p < 0.05$, ** $p < 0.01$

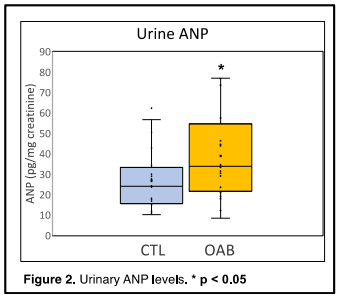

Figure 2. Urinary ANP levels. * $p < 0.05$

Conclusion: These results suggest that ANP may be linked to OAB by promoting imbalance between pro- and mature neurotrophins in bladder SMCs through increased production of MMP-9. The latter could suggest a new target for future therapies.

Research area: Overactive Bladder (OAB), Neurourology

P-16

Functional near infrared spectroscopy (fNIRS) reveals different patterns of neuroexcitation for women with and without overactive bladder during natural filling

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Introduction: Functional near infrared spectroscopy (fNIRS) is a non-invasive technique used to quantify prefrontal cortical oxyhemoglobin (O_2Hb) concentration, or neuroexcitation, during bladder filling and voiding. The objective was to compare prefrontal cortical O_2Hb levels during natural filling in female participants with and without overactive bladder (OAB).
Methods: Female participants with OAB were compared to controls without urinary urgency. Participants underwent continuous recording of prefrontal cortical O_2Hb using fNIRS during a validated oral hydration protocol. Simultaneous recordings of real-time sensation (0-100%) and "first desire" to void were completed. A period of "elevated urgency" was defined as the time from "first desire" to 100% sensation. Channels were sub-analyzed by cortical regions: right (9), left (9), and middle (6).
Results: Nineteen participants completed the study, including 9 with OAB and 10 without. The OAB group was older and had a higher BMI. The rate of change of O_2Hb concentration during the elevated urgency time period was significantly different between groups in all cortical regions (OAB 57.0± 22.2% vs controls 89.6± 11.5%, p=<0.001). The overall change in O_2Hb concentration from 0 to 100% sensation was significantly lower in the OAB group in all prefrontal cortical regions (OAB -0.02±0.86 vs control 1.32±0.35, p<0.001) as well as in 16/24 individual channels.
Conclusion: This study demonstrates that fNIRS cortical excitation during a period of elevated urgency is consistently lower in women with OAB as compared to controls. With additional research, fNIRS has the potential to detect unique neuroexcitation patterns in OAB patients. More importantly, although limited, this data supports the hypotheses that the prefrontal cortex plays an inhibitory role in voiding function and that there may be a lack of inhibitory control in women with OAB.

Research Area: OAB, Neurourology
Funded by [R21 DK128649-02, 1K12HD108269-01]

P-17

Barriers to adherence to overactive bladder for Hispanic women

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Introduction: Adherence rates to overactive bladder (OAB) treatments are low among the general population. Prior studies have demonstrated that OAB is more prevalent among Hispanic women compared to other ethnic groups. Hispanic women are under-represented in the OAB literature. The aim of this study is to analyze nonadherence to OAB therapy among Hispanic and non-Hispanic women, and identify potential barriers to treatment that may reduce disparities in care.

Methods: All patients greater than 18 years of age with an ICD-10 diagnosis of OAB that received treatment between 2018 and 2022 were included in this retrospective study. Race and ethnicity were self-reported by the patient. Interventions recommended, follow-up visits and completed treatments were tracked via chart review. Nonadherence was defined as the failure to initiate therapy within one year. This study was powered to detect a 50% difference in treatment nonadherence. Comparisons were made using Fisher exact test and Chi^2 test as appropriate for nominal variables, and Mann-Whitney test for continuous variables.

Results: Two hundred forty-one women (126 Hispanic vs 115 non-Hispanic) were analyzed. The two groups were similar regarding body mass index (BMI), parity, and insurance type. Hispanic women were younger at presentation (59.8+/-13.6 vs 68.7+/-13.6, $p<0.001$). Hispanic women were also more likely to have advanced pelvic organ prolapse (POP) ($p=0.03$) without difference in duration of symptoms, presence of stress incontinence, or OAB symptoms. There was no significant difference in therapy provided ($p=0.17$) and number of treatments completed ($p=0.93$) among the groups. Nonadherence to OAB therapy was more frequent among Hispanic women compared to non-Hispanic women (44.0% vs 20.9%, $p<0.0001$). After adjusting for age, BMI, primary language spoken, parity, insurance type, and treatment offered, Hispanic women were at 2.54-fold increased risk for nonadherence to OAB therapy (95% confidence interval: 1.30-4.97, $p=0.007$). A sub-analysis of only Hispanic women demonstrated that younger age ($p=0.05$), higher parity ($p=0.02$) and being underinsured ($p=0.05$) were associated with nonadherence to OAB therapy.

Conclusions: Nonadherence to OAB therapy was more frequent among Hispanic women despite no difference in treatment patterns. Younger age, higher parity and lack of insurance may be barriers to OAB treatment in the Hispanic population. These non-modifiable clinical correlates may indicate the need for more focused counseling for those with these risk factors. Nonetheless, further research is needed to elucidate the influence of social determinants of health on OAB treatment adherence.

Research Area: Overactive Bladder, Female Pelvic Medicine, Health Services Research

Sacral nerve stimulation for constipation in virtual and rodent colons

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Introduction: Sacral nerve stimulation (SNS) has been used to treat overactive bladder and fecal incontinence for over a decade. SNS modulates colon motility and could be an alternative to laxatives and conventional pharmaceuticals for the treatment of constipation. However, SNS has failed to treat slow-transit constipation in many clinical trials, and empirically adjusting the stimulation parameters has not produced clinically-relevant improvements in outcomes.
Methods: We hypothesized that bursts of SNS interleaved by quiescent periods would increase colonic transit more effectively than conventional, continuous SNS. We compared the effects of burst and conventional nerve stimulation on propagating calcium waves and intrarectal pressure in the isolated mouse colon and anesthetized rat, respectively. We developed a computational model of colonic motility and compared the effects of burst and conventional nerve stimulation on pellet velocity and colonic emptying under normal and slow transit conditions.
Results: Burst pelvic nerve stimulation evoked more frequent calcium waves in the isolated mouse colon than continuous nerve stimulation (Figure 1A,B). Continuous, but not burst, SNS increased resting rectal pressure in the anesthetized rat (Figure 1C,D). Continuous nerve stimulation did not increase pellet velocity or fecal pellet output in a computational model of slow transit. Optimal burst nerve stimulation with 8 Hz frequency, 20-30 s burst duration, and 40-80 s burst interval increased fecal pellet output by at least 50% in a slow transit model (Figure 1E,F).

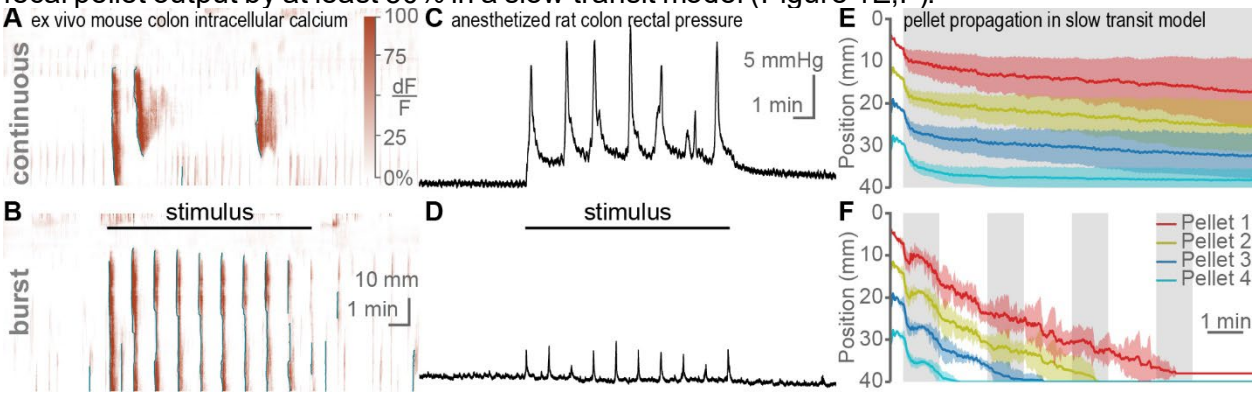

Figure 1: Responses to continuous vs. burst nerve stimulation in the virtual and rodent colons. (**A-B**) Spatiotemporal map of fluorescence from genetically encoded calcium indicators in the isolated mouse colon in response to (A) continuous and (B) burst pelvic nerve stimulation. (**C-D**) Rectal pressure recorded in the anesthetized rat in response to (C) continuous and (D) burst sacral nerve stimulation. (**E-F**) Mean (bold) and 95% confidence interval (shading) of pellet propagation in the slow transit model with (E) continuous and (F) burst stimulation (indicated by gray backgrounds). Each color corresponds to 1 of 4 pellets.
Conclusion: Burst nerve stimulation with optimized burst frequency, duration, and interval more effectively produced prokinetic motility than continuous nerve stimulation, suggesting that burst SNS may be a viable clinical treatment for severe and slow transit constipation. We acknowledge financial support from NIH R01 DK119795.

Research Area: Sacral nerve stimulation, therapeutic development, innovative technologies

AMPLIFY: Amplifying Sensation in Underactive Bladder (Work in Progress)

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Introduction: Incomplete emptying due to underactive bladder (UAB) is a poorly understood health condition that affects up to 40% of the aged population. Bothersome UAB symptoms for which patients seek care include pelvic pressure, urinary frequency, feelings of incomplete emptying, and/or slow stream. However, current management options are often associated with failure to resolve symptoms and poor quality of life. There is a need to clarify the pathophysiological mechanisms underlying UAB to improve outcomes. The objective of this study was to quantify sensory nerve sensitivity in adult women with UAB, and to compare urinary symptoms and urodynamic (UDS) parameters pre and post stimulation.

Methods: An abridged Lower Urinary Tract Dysfunction Research Network Comprehensive Assessment of Self-Reported Urinary Symptoms (LURN CASUS) questionnaire, measuring urinary frequency, bladder sensation, voiding effort, urinary flow, feelings during emptying, and quality of life, was administered before and 7 days after the study visit. Higher scores (range: 0-100) indicate greater severity of lower urinary tract symptoms (LUTS). The functional integrity of bladder and urethral sensory nerve fibers was assessed using current perception threshold (CPT) testing and was compared to published normative asymptomatic data (Kenton et al. 2007). CPT values were obtained using a forced choice paradigm by the method of levels. Continuous electrical stimulation was delivered via catheter electrode to selectively target the bladder (selection criteria: poor bladder sensation in CPT, first desire to void greater than 275 mL, or self-reported poor filling sensations) or urethra (selection criteria: poor urethral sensation in CPT, or detrusor pressure at max urine flow rate < 30 cmH_2O and max urine flow rate < 10 mL/s). UDS were performed after the stimulation session (15 min for urethra or 30 min for bladder) to assess bladder sensation and emptying function.

Results: Twelve subjects have participated out of our target of 20. Compared to normative asymptomatic women, the current amplitudes to elicit sensation were 352±189% higher in the bladder and 3,334±3,387% higher in the urethra. Compared to baseline, the average total CASUS score 7 days after stimulation decreased (improved) 7 points seven days after the study visit (53±11 vs 46±9). The subscale measuring frequency decreased by 3 (51±15 vs 48±10), effort decreased by 8 (48±23 vs 40±21), flow decreased by 13 (47±16 vs 34±7), emptying decreased by 6 (49±20 vs 43±19), and impact on quality of life decreased by 15 (77±17 vs 62±26) points. Compared to their pre-stimulation clinic UDS, the volume at first sensation increased by 58 mL, cystometric capacity increased by 52 mL, pressure flow voiding efficiency increased by 11%, max urine flow rate increased by 6 mL/s, and detrusor pressure at max urine flow rate increased by 14 cmH_2O after stimulation.

Conclusions: CPT testing confirmed poor urethra and bladder sensation in participants with symptoms of an UAB. Early findings suggest targeted electrical stimulation can acutely improve bladder symptoms and function. Future analyses will compare the effects of bladder to urethral stimulation, and future studies will determine if multiple stimulation sessions provide greater benefit.

Research Area: Voiding Dysfunction/Urinary Retention, Neurourology, Urodynamics
Funding: K01 DK120632 (EJG)

Specialized pro-resolving mediator Resolvin E1 does not inhibit caspase-1 driven inflammation in murine urothelial cells

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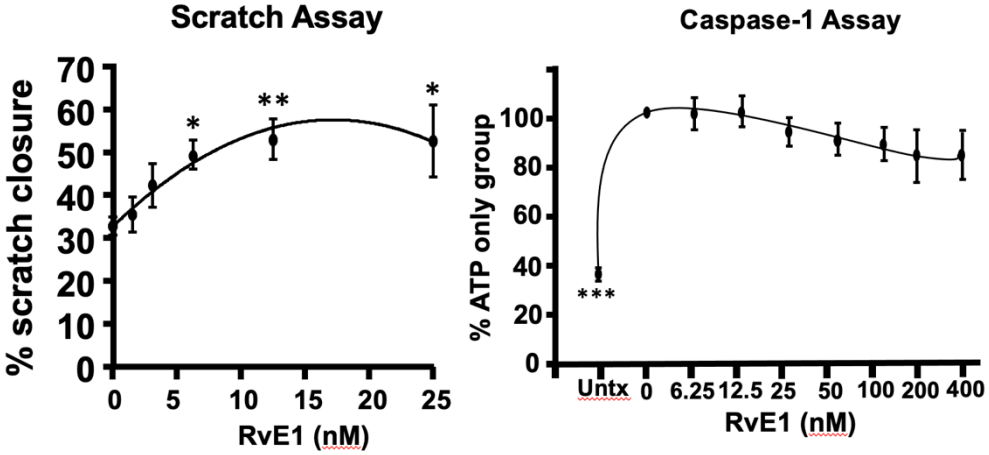
Introduction: Diabetes is a chronic inflammatory disease that affects 11.3% of Americans. One of its major complications is diabetic bladder dysfunction (DBD), which affects over 50% of patients with diabetes. Currently, DBD cannot be prevented by blood glucose control alone, and DBD has no specific therapies. Recent studies have shown a critical role for inflammation in DBD. Overall, inflammation has an initiation phase, mediated by factors such as the NLRP3 inflammasome, and a resolution phase, mediated by specialized pro-resolving mediators (SPMs). Our lab has shown the importance of the NLRP3 inflammasome, which activates caspase-1 to cleave pro-IL-1β and pro-IL18 into their active forms to commence initiation; however, no studies have examined a possible role for SPMs in resolving DBD. SPMs decrease inflammation by actions such as promoting the exit of immune cells from the tissue, increasing neutrophil apoptosis, and efferocytosis. They are particularly well known for promoting epithelial barrier repair and reducing the activity of the initiation phase, including NLRP3 activation. Taking an in vitro approach, we hypothesize in this study that the administration of an SPM, Resolvin E1 (RvE1), to urothelial cells will enhance barrier repair (scratch assay) and reduce NLRP3 activation (caspase-1 activity).
Methods: Urothelial cells were isolated from the bladders of male wild type mice using collagenase P. After incubating overnight, the cells were separated into three groups: 1)untreated, 2)ATP only treatment, and 3)ATP and varying concentrations of RvE1. A scratch and a caspase-1 assay were conducted. Data was analyzed using ANOVA followed by Student-Newman-Keul's post hoc test, $p<0.05$.
Results: ATP significantly increased caspase-1 activity in murine urothelial cells. RvE1 did not significantly reduce ATP-initiated caspase-1 activity, even at varying RvE1 concentrations.



Conclusion: RvE1 is not effective at decreasing in vitro NLRP3-driven inflammation in murine urothelial cells.

Research Areas: Bladder Inflammation, Diabetes

Resolvin E1 reduces diabetes-associated inflammation but does not improve urodynamic findings of underactive bladder in Akita male mice

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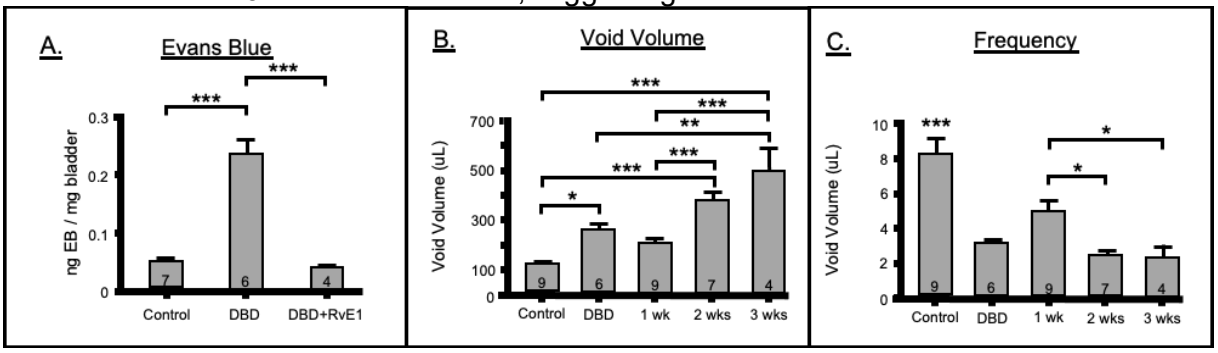
Introduction: One of the most common complications of diabetes is diabetic bladder dysfunction (DBD). Unlike other diabetic complications, DBD is not prevented with strict blood glucose control alone, suggesting other factors at work. Additionally, DBD has no current targeted therapies. Inflammation has been implicated as having a major role in both the onset and the progression of diabetes, which affects millions globally. Inflammation has two stages: initiation and resolution. The resolution phase is mediated by factors known as specialized pro-resolving mediators or SPMs. SPMs have previously been shown to decrease inflammation in murine bladders treated with cyclophosphamide or in a bladder outlet obstruction model. One SPM, Resolvin E1 (RvE1), has been shown to be effective in decreasing inflammation of epithelial surfaces, most notably, intestine and conjunctiva. This study proposes that RvE1 will decrease bladder inflammation and improve DBD signs in Akita mice, a type 1 diabetes model.
Methods: Beginning at 15 weeks of age, a time where DBD is well-established, Akita male mice were treated with daily i.p. injections of saline or 25 µg/kg RvE1 for one, two, or three weeks. Inflammation was assessed by the Evans Blue extravasation assay whereas bladder function was assessed by cystometry. Data was analyzed using ANOVA followed by the Tukey post hoc test, $p<0.05$.
Results: Saline-treated Akita male mice showed significant bladder inflammation, as measured by increased levels of Evans Blue extravasation. However, after only one week of RvE1 treatment Evans blue levels were restored to wild-type levels, demonstrating a complete resolution of inflammation. On cystometry, diabetic Akita mice showed increased void volumes and bladder capacity and decreased frequency when compared to wild type mice, demonstrating an underactive phenotype of DBD. After one week of RvE1 treatment these parameters were not significantly different. Surprisingly, after two weeks of RvE1 treatment indicators of underactivity actually increased and this was further exacerbated after 3 weeks of treatment, suggesting that RvE1 does not restore bladder function.



Conclusion: RvE1 treatment completely reversed diabetes-associated bladder inflammation but did not ameliorate signs of underactive bladder in Akita male mice, suggesting RvE1 would not be an effective treatment for diabetic bladder dysfunction.

Research Areas: Bladder Inflammation, Diabetes, Urodynamics

NLRP3 drives bladder denervation during diabetes in the male Akita mice

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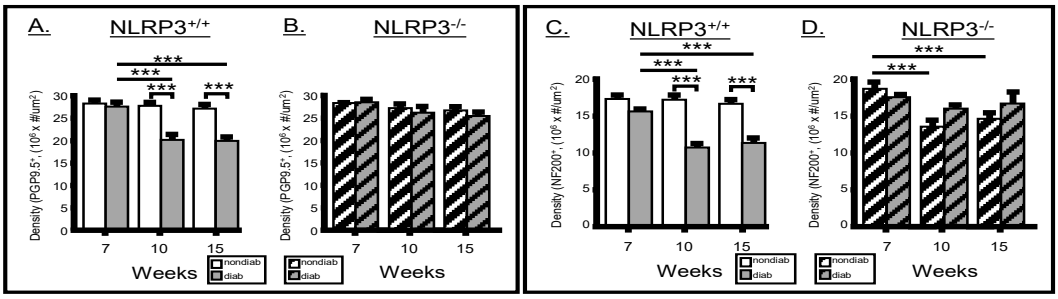
Introduction: Diabetic Bladder Dysfunction (DBD) is the most common diabetic complication. One of the most important changes to the bladder during DBD is a loss of nerves (denervation) leading to decompensation and eventual incontinence. Conventional thinking is that DBD is caused by inflammation and we have shown a critical role for the NLRP3 inflammasome in triggering this inflammation. Here, we hypothesize that NLRP3-induced inflammation is responsible for the denervation during diabetes. To investigate this hypothesis, we bred Akita mice (type-1 diabetes) with NLRP3$^{-/-}$ mice to create diabetic mice with NLRP3 absent. Using male mice, we quantitated the density of PgP9.5^{+} (a pan-neuronal marker) in the bladder wall over time. In addition, diabetics have a reduced sense of fullness which is carried to the CNS by the Aδ-fibers. Accordingly, we have also assessed the density of these fibers over time.

Methods: Four genotypes of mice were produced by the breeding core:

1. Nondiabetic NLRP3$^{+/+}$: Control mice. Mice homozygote for wild type Ins2 and homozygote for wild type NLRP3 gene.
2. Diabetic NLRP3$^{+/+}$: Diabetic controls. Mice heterozygote for the mutated Ins2 gene (the Akita mutation) and homozygote for the wild type NLRP3.
3. Nondiabetic NLRP3$^{-/-}$: NLRP3 knockout controls. Mice homozygote for wild type Ins2 with both copies of NLRP3 deleted.
4. Diabetic NLRP3$^{-/-}$: Diabetic knock out mice. Mice heterozygote for mutated Ins2 gene with both copies of NLRP3 deleted.

Male mice were examined at 7, 10, 15 weeks. Blood glucose was assessed by glucometer, inflammation by Evans blue dye extravasation. For nerve density bladders paraffin sections (5 um) were stained for PgP9.5 (a pan-neuronal stain) or NF200 (Aδ fibers), scanned, the bladder wall delineated and the number of stained cells counted. Density was then calculated.

Results: Blood glucose was greatly enhanced in the diabetic mice and this was not altered when NLRP3 was knocked out. Bladder inflammation was also enhanced in diabetics at 10 and 15 weeks compared to nondiabetic controls. Knocking out NLRP3 completely prevented inflammation in the diabetics at all time points. The density of PgP9.5^{+} neurons was statistically reduced by 10 weeks and remained there at 15. The NLRP3 knockout diabetics never showed this loss. Likewise, the density of NF200^{+} fibers exhibited a virtually identical pattern, being reduced at 10 and 15 weeks with the reduction absent in the NLRP3 knockouts.



Conclusion: Denervation in the bladder of the male Akita mice becomes apparent by 10 weeks and is dependent on the NLRP3 inflammasome.

Research Area: Inflammation, diabetes, neurourology

Male Akita diabetic mice develop underactive bladder independent of NLRP3 that can be prevented with blood glucose control

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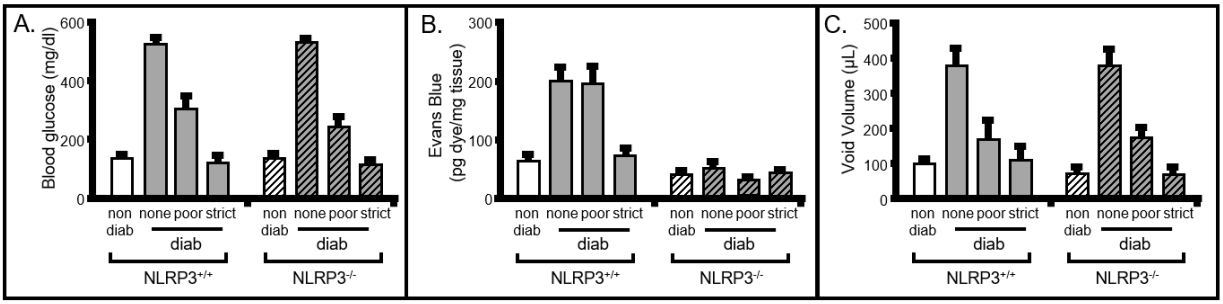
Introduction: Diabetic bladder dysfunction (DBD) can present with overactive bladder symptoms, underactive bladder symptoms (UAB), or both. While strict blood glucose (BG) control may be expected to reverse DBD, prior literature found no effects of intensive glucose control on symptoms. However, it is unclear if strict control, soon after the appearance of hyperglycemia, can prevent DBD. Moreover, nearly 50% of adult diabetics are poorly controlled despite therapy and it is unknown how this effects development of DBD. Thus, we sought to investigate the effect of early BG control (poor and strict) on the development of DBD. For this, we used male Akita diabetic mice (type 1) which experience severe hyperglycemia. Our lab has shown NLRP3-induced inflammation to be critical to the development of DBD in female Akita mice, which show milder hyperglycemia. Therefore, we also explored the possibility that NLRP3 plays a role in the development of DBD in the male .

Methods: Akita mice were bred with NLRP3$^{-/-}$ mice to create diabetics with NLRP3$^{+/+}$ or NLRP3$^{-/-}$ genotypes. These were stratified into well-controlled, poorly controlled, and uncontrolled BG groups based on insulin treatment (0.125 or 0.25 U/day). Mice were assessed at 15 weeks for BG (glucometer), inflammation (Evans blue), and bladder function (urodynamics).

Results: Average BG was elevated in diabetics, reduced in an insulin-dose dependent manner, and was not affected by the absence of NLRP3 (A). Diabetic/NLRP3$^{+/+}$ mice exhibited bladder inflammation which was prevented with BG control. This bladder inflammation was absent in NLRP3$^{-/-}$ mice at all BG levels (B). Diabetic mice also showed signs of underactive bladder (increased void volume, decreased frequency). This was strongly present in the uncontrolled group, attenuated in the poorly controlled diabetic mice but absent in the well-controlled diabetic group. Interestingly, deletion of NLRP3 did not affect voiding function (C).



Conclusion: Male Akita mice develop a UAB phenotype which can be prevented with glucose control. DBD develops independent of NLRP3-induced inflammation.

Research Area: Diabetes, bladder inflammation, voiding dysfunction/urinary retention

P-24

Diabetes increases cholinergic neurotransmitter-mediated contractions in the type 1 diabetic female Akita mouse due to inflammation mediated by the NLRP3 inflammasome

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Introduction: The most common complication of diabetes is diabetic bladder dysfunction which is driven in the female Akita diabetic mouse by inflammation mediated by the NLRP3 inflammasome. It is unclear how diabetes affects signaling pathways responsible for bladder contractility and if this is mediated by NLRP3. Using the type 1 diabetic female Akita model which develops an overactive bladder, we investigated effects on the cholinergic signaling pathways - the primary mechanism responsible for bladder contractions. To accomplish this we utilized *ex vivo* myography and western blot techniques to determine the impact of diabetes on the release of cholinergic neurotransmitters and muscarinic receptor activation in bladders of non-diabetic and diabetic mice ± the NLRP3 gene.

Methods: Diabetic Akita mice were crossbred with NLRP3$^{-/-}$ mice to yield 4 groups: non-diabetic and diabetic ± NLRP3 gene. Females were aged to 15 weeks when an overactive bladder phenotype is evident in diabetic NLRP3$^{+/+}$. For ex vivo myography, bladder strips with intact mucosa were used. Efferent neurotransmitter-mediated contractions were assessed using electric field stimulation (EFS) ± the muscarinic receptor antagonist atropine. Contractility to carbachol, a muscarinic receptor agonist, was also assessed (n=5-10 per group). Western blotting was used to measure protein expression of vesicular acetylcholine transporter (VACht; n=4 per group) in bladder smooth muscle.

Results At 15 weeks, diabetic NLRP3$^{+/+}$ mice have increased EFS contractility ($p<0.05$) but NLRP3 gene deletion prevented the diabetics from developing this pathology. EFS was repeated in the presence of atropine to determine the cholinergic contribution. In the presence of atropine, no significant differences between non-diabetic and diabetic animals were noted – indicating the increase in contractility at 15 weeks was due to upregulated cholinergic mechanisms. Interestingly, no changes in contractions to carbachol were noted. This indicates the increased contractility to EFS was due to an increase in acetylcholine release rather than to increased muscarinic receptor activity. Further confirmation of this mechanism is evident as higher levels of VACht protein expression (a marker of cholinergic nerve terminals) in the detrusor of diabetic NLRP3$^{+/+}$ mice. In the absence of NLRP3, diabetes did not impact bladder contractility to EFS and carbachol or impact VACht protein expression.

Conclusions: Diabetes increases cholinergic nerve terminals, neurotransmitter release, and bladder contractility in mice with an overactive bladder as a consequence of inflammation driven by NLRP3. This suggests an NLRP3 inhibitor may be an effective therapy to prevent overactive diabetic bladder dysfunction and eliminate the need for conventional anti-cholinergic treatments.

Funding: NIH-K12 DK100024; NIH-RO1 DK117890

Research area: Diabetes, bladder inflammation, voiding dysfunction / urinary retention

Extended treatment-dose antibiotic therapy versus low-dose prophylaxis for the management of recurrent urinary tract infections

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Introduction/Objective(s)
Veterinary data have shown medium-long-term effectiveness in preventing subsequent infections among canines and felines with recurrent urinary tract infection (rUTI) when treated with extended courses of treatment-strength antibiotics. In this study, we aimed to assess treatment efficacy over one year in women with rUTI receiving prolonged treatment-strength antibiotics compared to low-dose prophylactic antibiotic regimens.

Methods
A retrospective cohort study of adult women presenting with acute uncomplicated UTI between January 1, 2018 and October 1, 2020 meeting recurrent UTI criteria. Based on provider preference, women were offered either: 1) treatment-strength antibiotic therapy for 1 month; or 2) up to 7 days of treatment-strength antibiotic dosing followed by ≥3 months of prophylactic antibiotics. We excluded those with a complicated UTI The primary outcome was the presence or absence of a subsequent culture-proven UTI within 12 months of antibiotic treatment initiation. Multivariable logistic regression assessed differences in the primary outcome controlling for potential confounders.

Results
Of the 246 women included, 43 received extended treatment-dose antibiotics and 203 received ≥3 months of daily prophylactic antibiotics. Women who received 1-month treatment-dose antibiotics had a significantly lower risk of experiencing a subsequent UTI within 1 year when compared to those taking prophylactic antibiotics for ≥3 months (34.9% vs 59.6%; P<0.01). Time-to-event analysis showed a divergence in the survival curves beginning at 2 months favoring extended treatment dose antibiotics (Figure 1). This significant risk reduction was maintained in multivariable regression (aOR 0.42; 95% CI 0.20,0.89) controlling for age, estrogen use, anti-incontinence procedure, presence of rUTI with a single bacterial species, and diabetes.

Conclusion(s)
Women treated with a 1-month course of treatment-strength antibiotics had a significantly lower risk of subsequent UTI within 12 months compared to women receiving ≥3-months of daily low-dose prophylactic antibiotics after acute UTI treatment. Extended treatment-strength antibiotics may provide therapeutic benefit by clearing intracellular bacterial reservoirs through sustained higher-dose treatment while overall reducing cumulative antibiotic dose and duration. This innovative approach warrants evaluation in randomized trials.

Research area
Infections of the Urinary Tract
Female Pelvic Medicine
Clinical Outcomes Research

P-26

Prevalence of and Risk Factors for Bacteremic UTIs in Hospitalized Adults without Definitive Signs or Symptoms of UTI

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Background: IDSA guidelines recommend withholding treatment in patients with asymptomatic bacteriuria in the absence of systemic signs of infection. However, some of these patients may not be able to express symptoms either due to presence of indwelling catheter, complicated urologic anatomy, dementia, or altered mental status (AMS). Clinicians frequently treat bacteriuria in this population with antimicrobial therapy due to concern for sepsis. To determine treatment need, we reviewed prevalence and risk factors for bacteremic urinary tract infection (UTI) in a cohort of hospitalized inpatients without definitive signs and symptoms of a UTI.

Methods: This is a retrospective cohort study of inpatients with a positive urine culture who presented without definitive signs or symptoms of a UTI between 07/01/2017 and 06/30/2022 in 68 academic and community hospitals in Michigan. Signs and symptoms were obtained from medical record review 3 days before and after urine culture collection. Bacteremic UTI was defined as any positive blood culture growing at least one organism matching the urine culture. Risk factors for bacteremic UTI were assessed using multivariable logistic regression models with results expressed as odds ratios (ORs) for dichotomous variables and relative risks (RRs) for continuous variables.

Results: Of 11,793 patients meeting study criteria, 73.6% were female, median age 78.2 years, 41.8% had AMS, 33.8% had dementia, 15.6% had an indwelling urinary catheter, and 54.6% had complicated urologic history (e.g., urologic surgery). Of these, 166 (1.4%) patients developed bacteremic UTI. On adjusted analysis, male sex, hypotension, heart rate>90, urinary retention, fatigue, log of serum leukocytosis (1 log increase in serum WBC = 2.718 X Serum WBC), and pyuria with >25 white blood cells per high powered field (WBC/hpf) on urinalysis were associated with bacteremic UTI (Table). Older age, presence of an indwelling catheter, complicated urologic history, functional decline, AMS, dementia, and change in urine were not associated with higher odds for bacteremic UTI (Table). Of patients with AMS and no definitive signs or symptoms of a UTI, only 1.8% (89/4932) developed bacteremic UTI.

Table: Risk factors for bacteremic UTI in hospitalized older adults without definitive signs or symptoms of UTI, Multivariable Model

Variable (n=11,793)	N (%) unless specified	OR/RR	95% CI		P
Age (Median IQR)	78.2 (67.7-86.6)	1.01	1.00	1.03	0.07
Male sex	3114 (26.4%)	**1.45**	1.02	2.07	0.04
Hypotension (SBP<90)	888 (7.5%)	**1.79**	1.14	2.82	0.01
Heart rate >90 bpm	5407 (45.8%)	**1.68**	1.19	2.37	0.003
No AMS or Dementia	5299 (44.9%)	REF			
AMS (with or without dementia)	4932 (41.8%)	1.31	0.92	1.87	0.14
Dementia without AMS	997 (8.5%)	0.56	0.25	1.27	0.16
Change in urine color/character	2233 (18.9%)	1.42	0.97	2.10	0.07
Fatigue	3176 (26.9%)	**1.47**	1.04	2.08	0.03
Functional decline	947 (8.0%)	1.28	0.76	2.16	0.34
Urinary retention	927 (7.9%)	**1.79**	1.11	2.90	0.02
Indwelling catheter	1835 (15.6%)	0.93	0.61	1.43	0.75
Complicated urologic history	6440 (54.6%)	1.26	0.86	1.85	0.24
UA WBC/hpf 0-5	1441 (12.2%)	REF			
UA WBC/hpf 6-10	1263 (10.7%)	0.78	0.28	2.22	0.65
UA WBC/hpf 11-25	1765 (14.9%)	0.66	0.25	1.73	0.40
UA WBC/hpf >25	6577 (55.8%)	**2.47**	1.23	4.96	0.01
Log serum WBC		**3.88**	2.90	5.19	<.0001

Conclusion: Bacteremic UTI is relatively rare in hospitalized inpatients presenting with bacteriuria without symptoms of UTI. Predictors of bacteremic UTI included male sex, hypotension, tachycardia, urinary retention, fatigue, serum leukocytosis, and higher levels of pyuria (>25 WBCs/hpf) on urinalysis. Our findings provide a framework to risk stratify inpatients of older age who present with positive urine cultures but are unable to express signs or symptoms of a UTI.

Research Area: Infections of the Urinary Tract, Clinical Outcomes Research

P-27

Short- and long-term hypoxic response of bladder urothelial cells in vitro

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Introduction/Objectives: Bladder outlet obstruction (BOO) affects around 21.8% of adults over the age of 20 and is caused by a narrowing of urethra.[1] The obstruction results in mechanical and chemical changes in the bladder such as elevated pressure and blood flow disruptions (tissue ischemia) that can lead to chronic inflammation in the urothelium.[2,3] Our previous study demonstrated that exposure to elevated hydrostatic pressure (40 cmH_2O for 1 min) led to ATP-mediated NLRP3 inflammasome activation in rat urothelial cells *in vitro*.[4] Additionally, studies have shown that 48 hours of hypoxic exposure led to increased inflammatory cytokine release in bladder smooth muscle cells.[5] However, the role of for hypoxia in BOO-induced inflammation in the urothelium is unknown. Thus, we examined the short-term and long-term effects of hypoxia on NLRP3 activation in rat urothelial cell line (MYP3 cells) using a hypoxic culture media containing enzymes glucose oxidase (GOX) and catalase (CAT).
Methods: MYP3 cells were exposed to *in vitro* hypoxic conditions via a two-enzyme system for 2 or 6 hours. To validate the results obtained using the enzyme-based hypoxia model, a modular hypoxia chamber (flushed with a gas composed of 5% O_2, 5% CO_2 and balanced with nitrogen) was also used. Hypoxic media was prepared with 0.5 µg/mL GOX in phenol-red free F-12K media (VWR) with catalase (CAT, 120 U/mL) and HEPES (25 mM) to remove hydrogen peroxide, and to buffer pH, respectively. Cellular hypoxic response was examined through HIF-1α stabilization via immunostaining and nitric oxide (NO) release in the supernatant measured using Griess reagent assay (Fisher). Inflammasome activation was quantified through both extracellular levels of ATP and intracellular capsase-1 via an established protocol.[4] Data were statistically analyzed using either one-way or two-way analysis of variance (ANOVA) and appropriate post-hoc tests. P-values less than 0.05 were considered statistically significant.
Results: In the short-term (up to 2 hours), both enzyme and gas-induced hypoxia caused a significant increase in NO levels by MYP3 cells after 1 hour when compared to normoxic conditions. However, exposing MYP3 cells to hypoxia through neither method led to NLRP3 activation as intracellular caspase-1 levels were similar to the normoxic control. In contrast, exposure to long-term hypoxia (up to 6 hours) resulted in about a 1.6-fold increase in intracellular caspase-1 activity in MYP3 cells compared to control. Treatment with TXNIP inhibitor, verapamil, impeded the hypoxia driven increase in caspase-1.
Conclusion: The results of the present study indicate that longer exposure is needed to elicit an inflammatory response in urothelial cells even though with shorter time periods, a hypoxic response was observed. Furthermore, hypoxia-induced activation of the inflammasome may be driven primarily through ROS-TXNIP pathway, as opposed to purinergic signaling pathway observed in pressure-induced caspase activation. A better understanding of the mechanisms behind mechanically and chemically induced bladder wall inflammation opens opportunities to treat BOO-related bladder dysfunction.

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Research Area: Bladder Outlet Obstruction

Temporally Complex Inflammatory Networks in Animal Model Reveal Signatures for Interstitial Cystitis and Bladder Pain Syndrome Phenotype

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Introduction and Objective

Interstitial cystitis and bladder pain syndrome (IC/BPS) presents with symptoms of debilitating bladder pain and is typically a diagnosis of exclusion. Patients with Hunner's lesions and tissue inflammation on bladder biopsy have an increased likelihood of therapeutic success with anti-inflammatory drugs. However, identification of this subgroup remains difficulty due to the invasive nature of biopsies and the lack of surrogate biomarkers of IC/BPS. We modeled the dynamic evolution of inflammation in an experimental IC/BPS rodent model using computational biological network analysis of inflammatory mediators (cytokines and chemokines) in the urine. The use of biological network analysis allows us to identify urinary proteins that could be causative drivers of inflammation and potential prognostic biomarkers that could serve as therapeutic targets for the treatment of IC/BPS.

Methods

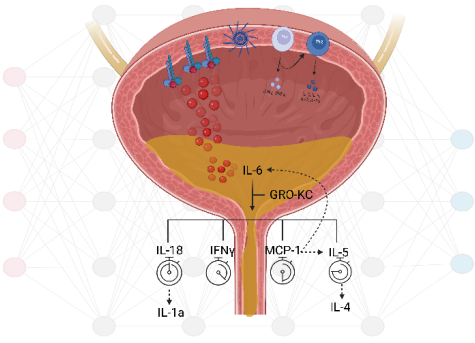
Rats subjected to cyclophosphamide (CYP) injection (150 mg/kg) were used as an experimental model for acute IC/BPS (n=8). Urine from each void was collected from the rats over a 12-hour period and was assayed for 14 inflammatory mediators using Luminex™. Time-interval principal component analysis (TI-PCA) and dynamic network analysis (DyNA), two biological network algorithms, were used to identify biomarkers of inflammation characteristic of IC/BPS over time.

Results

Compared to vehicle-treated rats, nearly all inflammatory mediators were elevated in the urine of CYP treated rats. TI-PCA highlights that at early timepoints, GRO-KC, IL-5, IL-18, and MCP-1 account for the greatest variance in inflammatory response. During the same early time interval, DyNA indicates a positive correlation between IL-4 and IL-1band TNFa and IL-1b. Analysis of TI-PCA and DyNA at later timepoints show the emergence of IL-5, IL-6, and IFNg as additional key mediators of inflammation. Furthermore, DyNA network complexity rises and falls before peaking at 9.5 hours following CYP treatment. This pattern of inflammation mimics the fluctuating severity of inflammation coinciding with IC/BPS flares.

Conclusions

Computational analysis of inflammation networks in experimental IC/BPS analysis expands on the previously accepted inflammatory signatures of IC by adding IL-5, IL-18, and MCP-1 to the prior studies implicating IL-6 and GRO as IC/BPS biomarkers. This analysis supports a complex evolution of inflammatory networks suggestive of the rise and fall of inflammation characteristic of IC/BPS flares. We suggest the need for a more nuanced and computationally advanced approach to defining inflammatory signatures characteristic of the flare-up of IC/BPS symptoms in humans.



Research Area: IC/BPS, bladder inflammation, data science/predictive analytics

Novel magnetic resonance imaging biomarkers of bladder wall composition in a preclinical model of interstitial cystitis/painful bladder syndrome

Suyama, Julie[1]; Statum, Sheronda[2]; Mohana-Borges, Aurea[2]; Ma, Yajun[2]; Anger, Jennifer[3]; Chung, Christine[2]; Alperin, Marianna[1,3,4]

[1]Department of Obstetrics, Gynecology & Reproductive Sciences, Division of Female Pelvic Medicine & Reconstructive Surgery, University of California San Diego, CA. [2]Department of Radiology, University of California San Diego, CA. [3]Department of Urology, University of California San Diego, CA. [4]Sanford Consortium for Regenerative Medicine, La Jolla, CA.

Introduction/Objective: Interstitial cystitis/painful bladder syndrome (IC/PBS) is a debilitating condition characterized by bladder pain, urinary urgency and frequency, and nocturia. Bladder wall hyperpermeability (BWH) due to dysfunction of the urothelial glycosaminoglycan (GAG) layer is implicated in disease pathogenesis. At present, there are no non-invasive diagnostic tests or reliable biomarkers for IC/PBS. Magnetic resonance imaging (MRI) represents a unique strategy for diagnosis, disease phenotyping, and monitoring treatment response in IC/PBS. T1ρ is a novel high-resolution non-contrast MRI technique deployed in musculoskeletal imaging to quantitatively assess tissue proteoglycan content, with no current application in the field of benign urology. Thus, the objective of this study was to validate T1ρ as an innovative non-invasive MRI tool to quantitatively assess bladder proteoglycan/GAG content in a preclinical BWH model of IC/PBS.
Methods: Three-month-old female Fisher rats (n=4) were catheterized transurethrally with an 18G angiocatheter and bladders were filled with 400μL of saline (control) or protamine sulfate (PS 10 mg/mL, BWH model) for 10 min. After 3 saline flushes, bladders were distended with 200μL of saline and rats were imaged in a 3T preclinical Bruker BioSpec scanner using T1ρ in addition to standard MRI sequences. Total MRI protocol length was 5.5 hours. Bladders were harvested upon completion of MRI from euthanized animals and processed for molecular analysis of GAG content using a dimethyl-methylene blue (DMMB) protocol. Molecular quantitation of bladder GAG concentration via DMMB was correlated with radiologic quantitative assessment (i.e., T1ρ relaxivity) to assess congruence between the two measures.
Results: PS-treated bladders trend towards a decrease in GAG content compared to saline controls (Fig. 1A). PS-treated BWH bladders demonstrate increased T1ρ signal intensity, representing a decrease in tissue proteoglycan content (Fig. 1B).

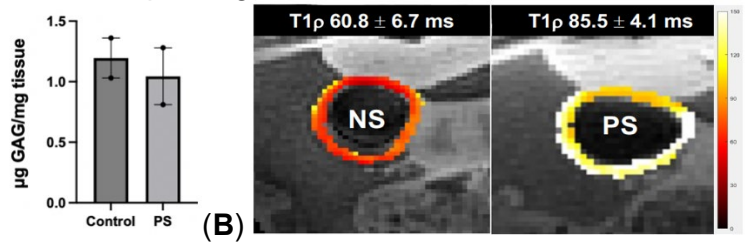


Figure 1. GAG concentration in control (normal saline, NS) and BWH (protamine sulfate, PS) rat bladders was measured biochemically using DMMB (**A**) and radiologically using T1ρ MRI (**B**). T1ρ signal increases as bladder GAG content decreases. N=2/group.
Conclusions: T1ρ MRI can be used for in situ quantitative assessment of bladder proteoglycan content. This high-resolution non-invasive MRI sequence represents a novel translational technique that may facilitate diagnosis of and treatment monitoring in IC/PBS based on quantitative tissue composition parameters. Validation of T1ρ as a reliable biomarker of bladder wall composition may provide an advanced and clinically relevant diagnostic tool to detect BWH in IC/PBS.

Research Areas: IC/PBS, Innovative technologies, Uroradiology

Urinary metagenomics (UMETA): A comparison of host cell depletion protocols and sequencing techniques for the urinary microbiome

Diaz, Nicole[1]; Rohrer, Gabrielle [1]; Karstens, Lisa[2]; Siddiqui, Nazema Y[1]

[1]Duke University Hospital, Durham, NC [2]Oregon Health and Science University, Portland, OR

Introduction/Objectives: There has been much interest in studying the urinary microbiome. Due to low microbial biomass and high levels of human DNA in urine, amplification methods such as 16S rRNA sequencing have typically been used. Shotgun metagenomics, where whole genome sequencing (WGS) is performed, is an attractive alternative since it provides species-level microbial data without amplification, as well as functional genetic information. However, WGS has been technically challenging and cost-prohibitive in low biomass urinary samples. We hypothesized that host cell or DNA depletion techniques would allow for successful shotgun metagenomics in urine. The goals of this study were: i) to compare the quality and resolution of urinary bacterial taxa after two different techniques to enrich bacterial DNA prior to sequencing; and ii) to compare recovered taxa from shotgun metagenomics after host depletion versus full-length 16S rRNA sequencing.
Methods: Urine samples were collected via clean catch voided urine in clinic or through transurethral catheterization in the operating room. Each sample was divided into 3 equal aliquots to allow for parallel processing. Samples underwent DNA extraction and eventual sequencing utilizing the following techniques respectively: 1) ultrasensitive long-read 16s rRNA gene sequencing offered through Loop Genomics (Loop-seq); 2) WGS after using the NEBNext methylation kit to deplete host DNA; and 3) WGS after a centrifuge technique to deplete host cells prior to DNA isolation. For all WGS data, additional host sequences were bioinformatically removed prior to analysis.
Results: We assessed urine specimens from 20 independent participants: 10 provided catheterized specimens and 10 provided voided specimens. Sequencing data were recovered from all samples processed with the centrifuge host cell depletion technique. When using the NEBNext technique to deplete host DNA, one catheterized sample failed to provide data. In contrast, the Loop-Seq technique failed to provide data for most samples, with only one catheterized and 5 voided samples having taxonomic information. Recovered taxa were similar in relative abundance after WGS with both host depletion techniques. Taxa were also generally consistent with those recovered through Loop-Seq. However, Loop-Seq showed the potential for contamination (see sample 021) or potential identification bias, as there was a lack of Actinobacteriota, which are known members of the urinary microbiome.

Conclusions: Shotgun metagenomics after host depletion is feasible in urine. The centrifuge technique for host cell depletion is more cost-effective but needs to be performed early in processing to remove host cells, while the NEBNext technique can be implemented to remove host DNA after DNA isolation. Both methods of host depletion produced similar taxonomic information, and higher quality data when compared to full length 16S rRNA sequencing.

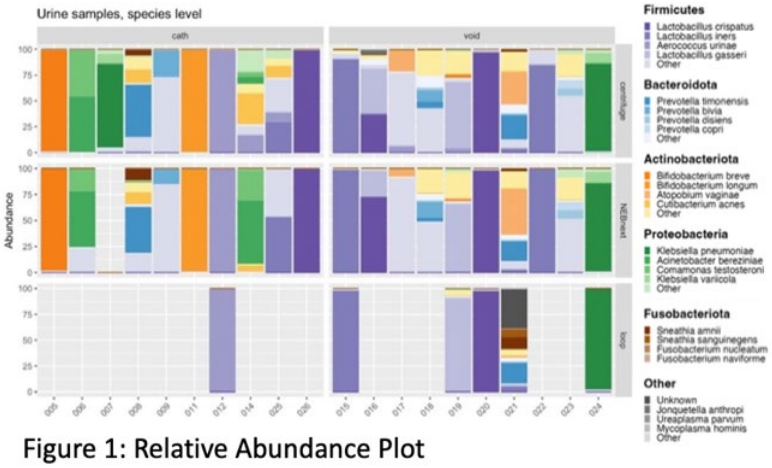


Figure 1: Relative Abundance Plot

Research Area: Microbiome

P-31

Integrating molecular epidemiology and mechanistic toxicology to unravel Chronic Kidney Disease of Unknown Origin (CKDu)

Merutka, Ilaria R[1]; Kolli, Ramya[1]; Chernick, Melissa[1]; Gunawardena, Kasun[1]; Dougherty, Kelsie A[2]; Babich, Remy S[3]; Jayasundara, Nishad[1];

[1]Nicholas School of the Environment, Duke University, Durham, NC. [2]College of Veterinary Medicine, North Carolina State University, Raleigh, NC. [3]Division of Environmental Health Epidemiology, Pennsylvania Department of Health, Harrisburg, PA.

Introduction: Chronic exposure to renal toxicants is an important but under-studied risk factor for the development of chronic kidney disease (CKD). Chronic Kidney Disease of Unknown Origin (CKDu) is an epidemic of early-onset, rapidly progressive kidney failure that is associated with exposure to agrochemicals. The underlying pathology of CKDu is lysosomal deficit within tubular epithelial cells, but the mechanisms that link agrochemical exposure to lysosomal pathology are not clear. Drinking water in CKDu endemic areas is polluted with the herbicide glyphosate (RoundUp) and toxic metals (As, Cd, V, and Pb) used in fertilizer; these are known mitochondrial toxicants and can impede lysosomal degradation. The widespread emergence of CKDu in agricultural communities worldwide highlights a gap in understanding the role of environmental contaminants on kidney disease onset and progression.

Methods: First, we characterize the urinary proteome of CKDu patients in Sri Lanka to better understand molecular epidemiology of this disease. Second, we use zebrafish as a mechanistic model to investigate how an environmental mixture of metals and glyphosate (M+G) undermines subcellular processes and deteriorates renal function. We profile transcriptional changes and mitochondrial function deficits from exposure to M+G and evaluate renal histology over time. Finally, we use zebrafish larvae to screen ~200 drinking water samples from Sri Lanka in order to directly relate chemical contamination of drinking water to mechanisms of renal toxicity.

Results: Altogether, our CKDu patient urinary proteome substantiates CKDu as a proximal tubular nephropathy. We report substantial overlap with CKDs resulting from mitochondrial deficiency as well as marked reduction in lysosomal enzymes and membrane components in CKDu patient urine. Using zebrafish as our mechanistic model, we find that exposure to the lab mixture M+G decreases mitochondrial respiration and stimulates mitophagy, downregulates lysosomal related genes, and induces progressive vacuolation in the proximal tubular epithelium. Finally, we show that exposure to drinking water samples from regions where CKDu is endemic impairs mitochondrial respiration more than those from non-endemic regions.

Conclusions: Across all avenues of investigation, we identify mitochondrial and lysosomal function as key subcellular processes which are impacted by drinking water contaminants and implicated in patients with CKDu. In ongoing work, we investigate the role that dysfunction of the mitochondrial-lysosomal axis plays in deteriorating renal function over time. In addition, we establish a pediatric cohort in Sri Lanka to identify risk factors and biomarkers for development of CKDu.

Research Area: Toxicology/Environmental Health

Mary Barbe, PhD
Fellow of the American Association of Anatomists
Center for Translational Medicine
Neural Science Department
Temple University School of Medicine

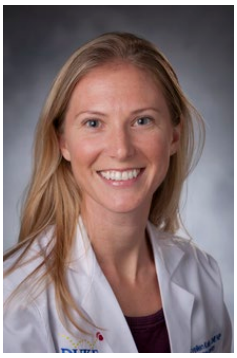
Dr. Barbe is a classically trained Anatomist with expertise in Neurobiology/Neuroscience and Musculoskeletal Biology with international recognition. She has 35 years of experience examining various aspects of peripheral and central neuroplasticity, ranging from changes occurring in the bladder (i.e., peripheral), spinal cord and brain after injury or gene knockout. One key focus of her lab since 2004 is examining methods of reinnervation of the bladder and related tissues after decentralization of the bladder after spinal root injury (with Dr. Ruggieri). She is now also examining the effects of bladder inflammation on bladder integrity and function. Another key focus of her lab since 2000 is studying the effects of repetition and force on tissues as a consequence of an upper extremity overuse injuries, using a unique operant rat model developed in her laboratory. Using this model, she has examined the effects of varying levels of repetitive and forceful work tasks on musculoskeletal and nervous system pathophysiology, focusing on injury and inflammation initially, and how these processes induced tissue degeneration and sensorimotor dysfunction. She is currently exploring inducers of tissue fibrosis and degeneration occurring with overuse, and effective interventions. She has over 230 peer-reviewed publications, and an H-index of 57. She is currently funded by the National Institute of Neurological Disorders and Stroke, the National Institute of Health's *National Institute of Arthritis and Musculoskeletal and Skin* Diseases, and the Department of Defense. Dr. Barbe serves on the KURe Advisory Board.

Christina Ching, MD
Clinical Associate Professor
Kidney and Urinary Tract Center
Nationwide Children's Hospital

Christina B. Ching, MD is an Assistant Professor in the Department of Pediatric Urology. She received her medical degree from Northwestern University Feinberg School of Medicine and completed her urology residency at Cleveland Clinic in Cleveland, OH. She then went on to complete a two-year fellowship in Pediatric Urology at Monroe Carell Jr. Children's Hospital at Vanderbilt University in Nashville, TN. Dr. Ching is interested in all aspects of urologic problems in children including urinary tract infections, hydronephrosis, urinary incontinence, hypospadias, kidney stones, ureteral reflux, spina bifida, and other complex pelvic and urinary conditions. She is trained in minimally invasive techniques of surgery as well as open. She has a strong interest in translational research and specifically how mechanisms of urothelial development and renewal are important in diagnosing, treating, and even preventing urothelial injury such as infection. Dr. Ching has an NIDDK-supported K08 award looking at the role of IL-6 signaling in UTI susceptibility.

Christopher S. Cooper, MD, FAAP, FACS
Senior Associate Dean for Medical Education
The University of Iowa Roy J. and Lucille A. Carver College of Medicine

Christopher S. Cooper, MD, FAAP, FACS, is Professor and Vice Chairman of Urology at the University of Iowa, Iowa City and serves as Director of the Pediatric Urology Division at the Children's Hospital. In addition, he has served as the Senior Associate Dean for Medical Education in the University of Iowa Carver College of Medicine since 2006. Dr. Cooper graduated from the University of Iowa College of Medicine and completed a two-year pediatric urology fellowship at the Children's Hospital of Philadelphia, Pennsylvania. His clinical research interests include vesicoureteral reflux (VUR), bowel and bladder dysfunction, neurogenic bladder, and hydronephrosis. In 2019, Dr. Cooper received the Societies for Pediatric Urology Clinical Research Prize for developing and patenting devices for home use in patients with neurogenic bladder that attach to a catheter to record bladder pressure and volume with intermittent catheterization. In 2022, the Urology Care Foundation of the American Urological Association recognized Dr. Cooper's career-long research contributions "to enhancing the treatment of children suffering with urologic conditions and improving their quality of life" with the John W. Duckett, Jr., MD, Pediatric Urology Research Excellence Award.



Maryellen Kelly, DNP, CPNP, MHSc
Assistant Professor
Division of Healthcare of Women and Children, Duke University School of Nursing
Pediatric Nurse Practitioner
Division of Urology, Department of Surgery, Duke Health

Dr. Maryellen Kelly is an Assistant Professor at Duke University and has been a pediatric nurse practitioner in urology since 2010. She obtained her MSN from Columbia University, Masters in health science clinical research from Duke University, and her DNP from the Univ. of Pittsburgh. Currently, she is funded by the NIH's NIDDK and NICHD centers, as well as the CDC for ongoing clinical and translational research related to spina bifida care and lower urinary tract conditions in children, namely overactive bladder, urinary tract infections, neurogenic bladder, and bowel. She is a manuscript reviewer for 8 journals and has over 20 publications. She sits on the Research Advisory Council for the Spina Bifida Association, is an Executive Board Member of the Pediatric Urology Nurses and Specialists Society (PUNS), and represents PUNS as an Editor for the Journal of Pediatric Urology.

Maragatha (Maggie) Kuchibhatla, PhD
Professor Biostatistics and Bioinformatics,
Psychiatry and Behavioral Sciences
Duke University School of Medicine

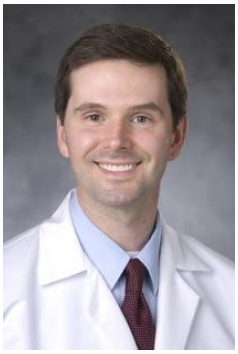
Dr. Kuchibhatla received her PhD from Texas A&M University. She is a Senior Fellow in the Center for the Study of Aging and Human Development. She is an expert in statistical research methodology, analysis of repeated measurements, latent growth curve models, latent class growth models, classification/regression trees, propensity score analyses and designing of clinical trials -- both treatment and non-treatment trials in various comorbid populations. Dr. Kuchibhatla serves on the KURe Advisory Board.

Brandon Lane, PhD
Assistant Professor
Department of Pediatrics
Duke University

Dr. Lane received his PhD in Human and Molecular Genetics from Virginia Commonwealth University. After completing postdoctoral training in gene therapy at UNC Chapel Hill, he joined the lab of Rasheed Gbadegesin at Duke University to study the genetics of pediatric kidney disease. While working under the mentorship of Dr. Gbadegesin, he was able to identify multiple single-gene causes of Nephrotic Syndrome as well as help define diagnostic criteria for genetic testing in these patients. The focus of Dr. Lane's current work is identifying podocyte-related disease mechanisms and therapeutic targets for the development of personalized medicine for patients with Nephrotic Syndrome.

Lori O'Brien, PhD
Assistant Professor
Dept. of Cell Biology and Physiology
University of North Carolina at Chapel Hill

Dr. O'Brien obtained her PhD in Biochemistry from the University of Wisconsin-Madison with a research emphasis on basic mechanisms of cell division. With an evolving interest in developmental biology, she then began her postdoctoral studies at Harvard and subsequently the University of Southern California where she investigated several aspects of renal development. This included the regulation of nephron progenitor cells during fetal development and how they differentiate into cells of the nephron such as podocytes. Dr. O'Brien's current lab at UNC-Chapel Hill continues to interrogate processes of kidney development such as vascularization and innervation of the kidney, how nephron progenitors transform into Wilms tumor, and the unique cell biology of podocytes.

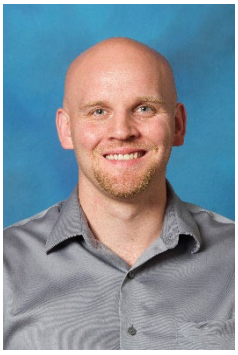


Jonathan C. Routh, MD, MPH, FAAP
Chief, Duke Center for Children's Surgery
Paul H. Sherman Distinguished Associate Professor of Surgery
Associate Professor in Pediatrics
Associate Professor in Population Health Sciences
Division of Urology, Duke University School of Medicine

Dr. Jonathan C. Routh is a pediatric urologist and health services researcher at Duke University School of Medicine, where he serves as the Chief of Children's Surgery and the Paul H. Sherman Distinguished Associate Professor (with Tenure) of Surgery, Pediatrics, and Population Health Sciences. His clinical & research interests include minimally-invasive surgery, complex urologic reconstruction (particularly in children with spina bifida and neurogenic bladder), surgical and non-surgical management of children with disorders of sex development, and pediatric urologic oncology. He is currently an Associate Section Editor for the *Journal of Urology*, the Chair of the Steering Committee for the Urologic Management to Preserve Initial Renal Function Protocol for Young Children with Spina Bifida (UMPIRE) study, and the co-PI at Duke for both UMPIRE and the National Spina Bifida Patient Registry (NSBPR). He has extensive experience as a mentor and currently serves as a formal mentor for both the KURe K12 and the UrogynCREST R25 programs; his list of mentees includes 4 undergraduate students, 10 medical students, 10 urology residents, 2 post-doctoral researchers, and 6 junior faculty members. In addition, he serves on the Advisory Committee for the Duke Urology K12 program and is the Co-Director of the Duke Research Development Course for Trainees.

Alison P. Sanders, PhD
Assistant Professor
Department of Environmental and Occupational Health
University of Pittsburgh School of Public Health

Dr. Sanders is an environmental health scientist with a background in engineering and environmental molecular epidemiology. Her research program examines how toxic chemical exposures and their mixtures alter early life kidney dysfunction in population-based studies. Dr. Sanders earned her PhD from the University of North Carolina at Chapel Hill and completed postdoctoral work at the Icahn School of Medicine at Mount Sinai. She is PI of an R00 award at the University of Pittsburgh and has founded and directed training and education programs for postdoctoral fellows, pre-graduates and 5th graders interested in science. Her research employs molecular epidemiology, toxicological, and computational approaches to investigate the effects of environmental exposures and their mixtures that may predispose susceptible populations including pregnant women and children to poor kidney function, chronic kidney disease (CKD) or CKD of unknown origin (CKDu).



Sunder Sims-Lucas, PhD
Associate Professor of Pediatrics
Associate Vice Chair of Education
University of Pittsburgh

Dr. Sunder Sims-Lucas is a basic research scientist, he is trained as an anatomist and developmental biologist. His research focuses on the formation of the kidney and the role of maternal stresses (including diabetes and malnutrition) on the formation of the kidney. Furthermore, his program focuses on acute kidney injury as well as the mechanisms that lead to predisposition to injury. The long-term goal of Dr. Sims-Lucas' research relates to the development of therapeutics to mitigate acute kidney injury. He has authored more than 70 publications and has an NIH R01 funded research program. He has a passion related to education and is Associate Vice Chair of Education at the Rangos Research Center and is integral in all levels of training including high school students, undergraduate students, graduate students and post-docs. Finally, he is also the Director of the Histology Core at the John G. Rangos Sr. Research Center.

Maryrose Sullivan, PhD
Research Health Scientist, VA Boston Healthcare System
Assistant Professor of Surgery, Brigham and Women's Hospital
Harvard Medical School

Dr. Sullivan's scientific interests have focused primarily on benign disorders of the bladder, including those related to outlet obstruction, diabetes, spinal cord injury and Parkinson's disease. Her research is aimed at uncovering mechanisms responsible for bladder function/dysfunction and urinary incontinence, with the ultimate goal of identifying targetable pathways for intervention and alleviating lower urinary tract symptoms. As a research scientist and biomedical engineer, her research projects exploit a number of multidisciplinary approaches to interrogate these pathways at the cellular, tissue and whole animal levels and include imaging, in vitro, ex vivo and in vivo techniques. With funding by the Department of Veterans Affairs and NIDDK, she has published numerous original articles, chapters and reviews on topics related to urinary incontinence, bladder contractility, bladder outlet obstruction, neurogenic and non-neurogenic detrusor overactivity, and diabetic bladder dysfunction. She has been fortunate to be involved in mentoring and supervising many urology residents, post-docs, medical students and junior faculty. She is also an active member of the AUA, SUFU, SPR and ICS, and serves on the editorial board of several urology focused journals. Dr. Sullivan serves on the KURe Advisory Board.

Philip J. Walther, MD, PhD, MBA, FACS
Professor of Surgery/Urology
Associate Professor of Experimental Pathology
Duke University

Dr. Walther received his MD-PhD as a Duke MSTP trainee; his urologic residency at UCLA; an American Cancer Society junior faculty fellowship at Duke; and subsequently an MBA from Duke's Fuqua School of Business (health care management). His lab research interests have been: 1) Developmental GU onco-therapeutics using human xenograft-supported GU tumors (primarily bladder) 2) the genomic elucidation of the role of oncogenic HPV genotypes with lower GU cancers (bladder, penis, and urethra). He served as Chair, GU Surgery Subcommittee of the NIH-funded cooperative study group-CALGB. He was the Site PI at Duke for the first NIH-sponsored multi-institutional study of immune-therapeutics of renal cancer using high-dose interleukin-2, and served as PI of a R21-funded grant to initiate an institutional research program in Prostate Cancer (seeding startup seed research grants). He also was PI of a VA-based epidemiologic effort (with Community Medicine) in the study of relevant black vs. white genomic differences associated with Prostate Cancer occurrence. Finally, he served on the Study Committee of a 7 year, 35000+ man NIH-sponsored nutritional intervention Prostate Cancer prevention study (Vitamin E vs. Selenium -SELECT). Dr. Walther serves on the KURe Advisory Board.