National Institute of Diabetes and Digestive and Kidney Diseases

National Institutes of Health

BPH and Male LUTS: Intersection Between Pathology and Disease

Virtual Meeting

March 30−April 1, 2022

SUMMARY REPORT

WEDNESDAY, MARCH 30, 2022

Opening Remarks and Objectives

Tracy Rankin, Ph.D., National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), National Institutes of Health (NIH)
Robert Star, M.D., NIDDK, NIH

Dr. Tracy Rankin welcomed participants and invited Dr. Robert Star, director of the Division of Kidney, Urologic, and Hematologic Diseases (KUH), to provide opening remarks.

Dr. Star commented on the purpose of the meeting, which is to bring together researchers and clinicians to discuss the molecular and clinical heterogeneity of benign prostatic hyperplasia (BPH) and lower urinary tract symptoms (LUTS) and to develop a consensus regarding directions for improved BPH/LUTS therapies. New precision approaches and tools will provide patients with the correct treatment at the right time. Dr. Star thanked the meeting organizers for their efforts and the participants for joining the workshop.

Dr. Rankin acknowledged the organizing committee: from the NIH, Dr. Julie Barthold (NIDDK), Dr. Candace Kerr (National Institute on Aging [NIA]), Dr. Ziya Kirkali (NIDDK), and Dr. Christopher Mullins (NIDDK); and from the academic community, Dr. James Brooks (Stanford University), Dr. William Ricke (University of Wisconsin–Madison), and Dr. Claus Roehrborn (The University of Texas Southwestern Medical Center).

Dr. Rankin asked participants to consider healthy prostate function and reasons for its predisposition for pathology. She provided an overview of the male urogenital system and highlighted unique aspects of prostate physiology. Dr. Rankin described a case study of an 81-year-old male with elevated serum creatinine who was diagnosed with bilateral hydronephrosis, which was likely due to prostatic obstruction. The patient elected to undergo transurethral resection of the prostate (TURP) followed by post-surgery treatment with an alpha-blocking drug. Dr. Rankin highlighted the patient's post-treatment difficulties with irregular urination patterns and explained that this patient is her father, who has given her permission to share his story.

Participants were provided a list of the breakout session topics and related questions before the workshop. Dr. Rankin noted that breakout group leaders would facilitate and organize the discussion report-outs to the audience. She encouraged participants keep the breakout session topics and questions in mind during the meeting. Dr. Rankin expressed her hopes that this NIDDK workshop and its discussions will unearth new questions and avenues of discovery to help all patients with BPH/LUTS.

SESSION 1: LANDSCAPE—RELATIONSHIP BETWEEN BPH AND MALE LUTS

Overview

Claus Roehrborn, M.D., The University of Texas Southwestern Medical Center

Dr. Roehrborn provided an overview of male LUTS and BPH. The first BPH guidelines were triggered in the 1980s by demands from the Agency for Health Care Policy and Research (currently known as the Agency for Healthcare Research and Quality). The first evidence-based BPH guidelines were published in 1994 and ushered in a period of unprecedented BPH research and discovery. Dr. Roehrborn noted that BPH is defined by a characteristic histology, whereas LUTS is defined and measured clinically using such tools as the International Prostate Symptom Score (IPSS). He listed several related pathologies—including benign prostatic enlargement (BPE), benign prostatic obstruction (BPO), and bladder outlet obstruction (BOO)—which can be symptomatic or not, and also overlap. BPH prevalence increases linearly as men age, and Dr. Roehrborn highlighted the remarkably similar prevalence of both BPH and LUTS in studies from around the world. He explained that men experience a host of storage, voiding, and post-micturition symptoms, with nocturia being the most common complaint. Dr. Roehrborn pointed out that unrecognized and unpresented symptoms likely represent a large proportion of the true prevalence of urologic conditions in any population. He noted that an aging world population will require more care and discussed future urology workforce considerations.

Dr. Roehrborn described the evolution of BPH symptom assessments. Dr. Michael J. Barry and colleagues developed the American Urological Association (AUA) Symptom Index, which was published in *The Journal of Urology* in 1992. When combined with a quality-of-life score, this metric is referred to as the IPSS. Although the IPSS performs well and is used worldwide, it has been subject to criticisms—most notably that it does not include questions related to incontinence. The NIDDK established the Symptoms of Lower Urinary Tract Dysfunction Research Network (LURN) with the main goal of developing an assessment tool that would address the deficiencies of the IPSS. LURN Symptom Index 10 (LURN SI-10) and LURN SI-29 scores were published in 2019 and correlate well with the IPSS; however, the improved LURN questionnaires have not been adopted widely by clinicians.

Dr. Roehrborn listed treatments for moderate-to-severe male LUTS associated with BPH (e.g., watchful waiting, medical therapy, minimally invasive therapies, surgical therapies). He commented on the selection bias inherent in population studies, registries, cohorts, and randomized control trials (RCTs). Most studies are limited to patients between the ages of 40 and 80 experiencing a narrow range of clinical symptoms. This bias might be acceptable for clinical trials but restricts what currently is known about BPH. Such conditions as bladder detrusor underactivity, nocturia, and intravesical lobe protrusion remain incompletely understood. BPH is a mix of stromal tissues, which respond to adrenergic alpha blocking drugs, and glandular epithelial tissues, which are under androgen influence and respond to treatment with 5-alpha-reductase inhibitors (5ARIs). Clinicians and researchers need to better understand the tissues that are being treated. Only recently has the field begun to appreciate unique roles for inflammatory cell infiltrates and interstitial fibroblasts in BPH.

In closing, Dr. Roehrborn called attention to a theoretical “window of opportunity” for BPH treatment. For example, shifting the treatment window for BPH to earlier in life would result in better patient outcomes.. Dr. Roehrborn summarized challenges for this meeting and for the next generation of clinicians and researchers, including the need for effective workup and treatment algorithms, the ability to target newly identified cell types, and the inclusion of more diverse patients and more clinical information in studies.

Discussion

- Dr. Ricardo Gonzalez (Houston Methodist Research Institute) commented on the utility of voiding diaries to bladder clinicians and how voiding is difficult to quantify in patients with LUTS with bladder noncompliance. Dr. Roehrborn responded that "pendulum swings" (i.e., changes in thoughts over time) are common over the course of medical history. Clinicians have swung from historical overuse of TURPs to being hesitant to provide surgical therapies. Quantitative measures, such as voiding diaries, will be useful in moving away from the current dependence on patient symptom reporting.
- Dr. Robert Matusik (Vanderbilt University Medical Center) asked about the histological definition of BPH. Dr. Roehrborn answered that, at the moment, BPH can be defined only using a biopsy. He mentioned Dr. Douglas Strand's (The University of Texas Southwestern Medical Center) efforts to categorize BPH using magnetic resonance imaging (MRI).
- Dr. Brooks wondered whether preventive treatments for BPH would differ from therapeutic treatments. He asked how BPH parameters could be assessed in a minimally invasive way during an earlier treatment window. Dr. Roehrborn responded that the UroCuff—a noninvasive diagnostic tool to evaluate bladder function, pressure, and urine flow—was one option. Bladder wall thickness might be assessed with ultrasound imaging.
- Dr. Scott Bauer (University of California, San Francisco [UCSF] and San Francisco Veterans Affairs Medical Center) noted that not all patients with BOO experience LUTS. He asked how confident clinicians can be about inferring causality when multiple conditions overlap. Dr. Roehrborn agreed: not all patients with obstruction have symptoms, not all patients with obstruction have an enlarged prostate, not all patients with enlarge prostates are obstructed, and none of these pathologies completely coincide with symptoms. The overlap and interplay between these conditions is a major challenge to resolve.
- Dr. Star asked the group whether clinical urologists find the IPSS useful, and several clinicians commented positively on its utility. Dr. Gonzalez noted that third-party payers require the IPSS before paying for certain procedures.

Epidemiology of BPH/LUTs—What Have We Learned from Current Cohorts?
Marvin Langston, Ph.D., Kaiser Permanente

Dr. Marvin Langston described his interest in creating personalized approaches for treating BPH/LUTS and provided an overview of challenges in this area. He pointed out that knowledge of BPH/LUTS risk factors has not kept pace with advances in treatments. The assumption that BPH and LUTS are natural consequences of aging has precluded epidemiological studies of these conditions. The lack of quantitative measures for relevant parameters also has been a challenge. Dr. Langston pointed out that the causes of LUTS in men are multifactorial and involve many organs outside of the prostate. BPH/LUTS outcomes have been assessed in various ways in population health research (e.g., histological analysis of prostate tissue, urodynamic analysis, need for BPH surgery, acute urinary retention). Researchers utilize varying definitions of BPH/LUTS and different modes of survey administration. They face barriers (e.g., cultural, psychosocial, economic) that prevent accurate symptom reporting and often encounter responder bias. Advanced medical record phenotyping and cluster analysis methods are being developed, but generating comparable estimates across epidemiological studies on BPH/LUTS remains difficult.

Despite these challenges, it is clear that the prevalence of BPH/LUTS increases with age. Dr. Langston pointed out that nocturia is the most common symptom and that symptom progression might be dynamic across time. He highlighted racial variation in BPH/LUTS, noting that that Black and Hispanic men display higher rates of BPH/LUTS incidence and prevalence. Heritability of BPH is likely high and

Dr. Langston discussed candidate gene studies and genome-wide association studies (GWAS) investigating genetic susceptibility variants. Additional studies with large sample sizes are necessary for further analysis of the genetic underpinnings of this pathology. The growing field of BPH/LUTS etiology research has uncovered such additional risk factors as metabolic syndrome, obesity, diabetes, diet, physical activity, inflammation, and hormone levels. Additional epidemiological studies are necessary to confirm these associations. Dr. Langston noted that comorbidities associated with BPH/LUTS largely are metabolic and vascular conditions. He highlighted evidence from several population studies showing that erectile dysfunction is a condition that likely coexists with BPH/LUTS and noted that a common etiology or connection should be investigated. Dr. Langston summarized future needs in the field of BPH/LUTS epidemiology, including well-designed etiology studies and evaluations of the contributions of psychosocial factors and social determinants of health.

Discussion

- Dr. Kevin Abbott (NIDDK) commented that the side effects of current treatment options constitute a barrier to preventive treatment models. More benign treatment options are necessary. Dr. Rankin recounted that her father discontinued his treatment because of unpleasant side effects.
- Dr. Gonzalez noted that incontinence is one of the main reasons that older adults enter resource-heavy care facilities. He pointed out that, in addition to treating symptoms, clinicians aim to prevent major lifestyle changes. He called for better diagnostics for identifying patients who would benefit from early interventions.
- Dr. Ricke commented on the genetic linkage studies, noting that genetic relatives also share lifestyle and environmental factors that might play a role in determining BPH/LUTS risk. Dr. Langston responded that BPH/LUTS candidate gene studies and GWAS still are in the early stages of development. He added that population-based studies can capture lifestyle factors very well, but methods for measuring BPH/LUTS outcomes and parameters must still be refined.
- In response to a participant's question about vocational risk factors, Dr. Langston answered that BPH/LUTS etiology data are scarce, but it does not appear that jobs requiring long periods without urination are associated strongly with BPH/LUTS.

LUTS Clinical Workup and Approach to Treatment
Brian Helfand, M.D., Ph.D., University of Chicago

Dr. Brian Helfand presented on the clinical evaluation of LUTS and approaches to treatment from his perspective as a urologist. He noted challenges in the field, including the overreliance on self-reported symptoms, the lack of quantifiable biomarkers, and a prostate-centric view that automatically attributes urinary dysfunction to prostate enlargement. He pointed out that prostate volume does not correlate well with symptom severity as measured by the IPSS. Dr. Helfand associated this lack of understanding of the etiology of LUTS with high treatment failure rates. Half of patients report unsatisfactory outcomes after medical treatment, and up to 35 percent of patients exhibit persistent or recurrent LUTS after surgery.

Dr. Helfand described the complex etiology of LUTS. He listed urologic causes and provided evidence from United Kingdom Biobank and LURN studies for endocrine, inflammatory, psychological, dietary, sleep, and neurological contributions to LUTS symptoms. Data from the Complementary and Alternative Medicine for Urological Symptoms trial demonstrated an association between sleep disturbance and both daytime and evening urinary symptoms. Associations between sleep apnea, diabetes, inflammation, and LUTS were observed in National Health and Nutrition Examination Survey Longitudinal Study data. Dr. Helfand described the results of a recent study of inflammatory diseases and urinary symptoms in more than 1 million patients. Men with autoimmune or inflammatory diseases exhibited an increased

prevalence of BPH compared with the general population. Notably, men who were diagnosed with and treated for an immune disease were less likely than reference patients to develop BPH. Dr. Helfand presented data from mice and humans showing that immune modulation by inhibition of tumor necrosis factor alpha (*TNFα*) can prevent BPH.

Dr. Helfand explained how these new associations should be factored into patient evaluations. Current BPH guidelines call for detailed patient histories to be obtained. Known comorbidities should be documented and identified as possible causes of LUTS/BPH. Dr. Helfand reviewed the advantages of the LURN symptom index, which captures information about incontinence, post-micturition symptoms, and urinary pain. He emphasized that the LURN score better reflects patient discomfort regardless of the type of incontinence analyzed. Dr. Helfand expressed his belief that asking patients to identify their most bothersome symptom provides insight into BPH/LUTS evaluation. For example, patient complaints of weak streams or incomplete emptying suggest obstruction, whereas patient complaints of urgency suggest overactive bladder (OAB). Although medication can be prescribed for patients complaining of nocturia, it is reasonable to assess these patients for sleep disorders during workup. Men with LUTS also should be evaluated for such potentially modifiable factors as fluid intake, medication use, obesity, diabetic control, anxiety, and depression. Preliminary studies in patients with both LUTS and confirmed sleep apnea indicate that therapy addressing both conditions is more effective at treating urinary symptoms. Dr. Helfand encouraged clinicians to evaluate non-urological factors that contribute to LUTS and address them independently or alongside BPH.

SESSION 2: PROSTATE BIOLOGY AND PATHOBIOLOGY

Prostate Development

Chad Vezina, Ph.D., University of Wisconsin–Madison

Dr. Chad Vezina provided an overview of prostate development and presented on Linking Prostate Development to BPH/Lower Urinary Tract Disease (LUTD). He credited Dr. John McNeal with recognizing the connection between prostate development and BPH in the 1970s. Dr. Vezina explained that the prostate derives from a transient structure known as the urogenital sinus (UGS) before 13 weeks of gestation and pointed out that the developing UGS is similar between humans and mice at the histological and molecular levels. Dr. Vezina presented a video of UGS development and highlighted ductal bud and branch origins. He explained that the anatomic distribution of prostatic buds in the fetus determines the anatomic distribution of the main prostatic ducts in the adult. He added that prostate development is organized into stages (e.g., pre-bud, budding, branching, adult, advanced age), each with its own window of responsiveness that is regulated by hormones, growth factors, and epigenetic factors. Dr. Vezina explained that androgens from the prostatic stroma provide instructive signals to the epithelium, thereby activating budding and controlling its patterning.

Dr. Vezina listed several remaining questions in the field of prostate development. It is not clear how similar the molecular anatomy of human prostate development is to prostate development in mice or the progression of BPH. Additionally, it is unknown whether the microanatomy of BPH nodules involves new budding from the urethra, or whether the reawakening branching emerges from a single duct or multiple ducts. The extent to which events during the fetal period contribute to BPH/LUTD risk also is unclear. Dr. Vezina provided several examples of fetal exposures that increase the risk of disease later in life. Depending on the dosage, fetal estrogen exposure can either promote (low dose) or inhibit (high dose) prostate development. Fetal estrogen exposure also can worsen urinary retention in certain strains of adult mice and has been shown to drive changes in prostate methylation and RNA expression in adult rats. Fetal dioxin exposure permanently increases prostatic noradrenergic axon density and can alter adult mouse voiding behavior. Dr. Vezina asked participants to consider how events that occur during early life stages contribute to BPH and LUTD.

Discussion

- Dr. Rankin asked whether the stromal effects of BPH could result from the reawakening of developmental processes in prostatic buds. Dr. Vezina responded that the reawakening hypothesis could explain both stromal and epithelial nodules. Developmental signals determine stromal aspects of the prostate, and aberrations in these signaling pathways during development could predispose the microanatomy of prostate to disease.

Human Prostate Cellular Anatomy and Relationship to BPH Pathology
Doug Strand, Ph.D., The University of Texas Southwestern Medical Center

Dr. Strand provided an overview of the relationship between BPH pathology and the cellular anatomy of the prostate. His research group is interested in how prostate cell types change in abundance and in molecular characteristics during disease. Dr. Strand compared sections of a healthy prostate with a prostate undergoing early BPH and noted that cellular expansion was limited to the transition zone. He stated that a better understanding of the cell types involved in transition zone remodeling would provide insight into patient response to drug treatments. To address this question, Dr. Strand's group performed single-cell RNA sequencing (scRNA-seq) analysis on dissected prostates collected from healthy young organ donors. Two populations of uncharacterized cells were observed after cluster analysis of prostate cell types; these populations were found in the proximal and transition zones of the prostatic urethra and associated ducts. Gene expression analysis of these cells provided a cellular definition of the transition zone: a transition from urethral club and hillock epithelial cells to prostate-specific NK3 homeobox 1 (*NKX3.1*)–positive luminal cells.

Dr. Strand described the morphological progression underlying clinical BPH. Histological expansion of the transition zone—with epithelial gland cells growing into enlarged stromal nodules—can be observed in early BPH. End-stage BPH is heterogeneous across patients; advanced BPH can be purely glandular, purely stromal, or a mixture of the two populations. Dr. Strand hypothesized that these phenotypes could affect patient responses to treatment. To investigate stromal cell types present in each BPH phenotype, his group characterized stromal fibroblasts and smooth muscle cells present in normal human prostates. These cells were subcategorized into three smooth muscle subtypes and two novel fibroblast subtypes: apolipoprotein D–positive peri-epithelial fibroblasts and complement C7–positive (*C7*+) interstitial fibroblasts. Both glandular and stromal BPH phenotypes exhibited an expansion of interstitial fibroblast cells, with no apparent change in peri-epithelial fibroblasts. To determine whether interstitial fibroblasts reawaken to induce glandular branching into stromal nodules, Dr. Stand and his laboratory evaluated RNA from *C7*+ fibroblasts growing within and outside BPH nodules. Although overall gene expression was similar between both cell types, several developmental genes were upregulated in the fibroblasts growing within nodules. Dr. Strand postulated that, in addition to the increased number of interstitial fibroblasts, an increase in developmental signaling molecules causes hyperplasia.

Dr. Strand described how the cellular composition of BPH might affect patient treatment outcomes. Patient responses to 5ARI treatment are heterogenous; some prostate areas show improvement as evidenced by glandular atrophy, but some areas do not change at all. His group performed spatial transcriptomics of control and 5ARI-treated BPH tissues and observed prostate luminal plasticity on treatment. Some glandular cells adopted a urethral club cell-like phenotype that correlated with atrophied morphology in response to 5ARI administration. Dr. Strand noted that resistant glands mainly are found inside nodules and that these nodules might represent a cellular 5ARI-resistant phenotype. He illustrated how MRI can be used as a noninvasive clinical predictor of cellular phenotypes and how prostate-specific antigen (PSA) levels correlate with glandular cell composition, regardless of prostate size.

Discussion

- Dr. Rankin asked whether transcriptomic profiles of transition zone cells revealed anything about their function. Dr. Strand answered that prostatic urethra cells are similar to trachea and lung epithelial cells. These cells function in immune and anti-inflammatory roles, secreting such molecules as defensins. He added that although peri-epithelial fibroblasts express metabolic genes to lower oxidative stress, interstitial fibroblasts have a reparative profile.
- In response to a question from Dr. Matusik regarding whether club cells were progenitor cells for luminal cells, Dr. Strand explained that this is unclear. Lineage tracing experiments of urethral luminal cells in mice have shown that prostate-specific *NKX3.1*-positive luminal cells are not derived from club cells. He noted that many so-called prostate progenitor markers expressed in urethral club and hillock epithelial cells are merely markers of urological epithelia.
- Dr. Brooks asked whether it was possible to determine if *C7*+ stromal fibroblasts are drivers of BPH or merely passengers. Dr. Strand responded with his belief that the results from the spatial transcriptomic experiment (comparing *C7*+ fibroblasts inside and outside nodules) was evidence that these cells were driving active growth.
- In response to a question from Dr. Laura Pascal (University of Pittsburgh School of Medicine) about the difference between normal aging and changes in response to BPH, Dr. Strand pointed out that lower testosterone levels associated with aging result in the conversion of prostate luminal cells to a prostatic urethra phenotype. He agreed that normal aging phenomena (e.g., alterations in response to changing androgen levels) must be separated from pathology. Dr. Pascal asked about the role of the immune system in the aging urethra. Dr. Strand noted the high incidence of urinary tract infections in older women, possibly indicating that these urethral cells are defective.
- Dr. Roehrborn commented that such physiological measures as altered PSA levels and prostate volume changes can be measured after 5ARI treatment. These changes are variable and do not necessarily correlate with clinical responses (e.g., IPSS score) as they currently are understood. This lack of correlation has not been investigated adequately.
- Dr. Rankin asked whether androgen levels within tissues were assessed. Dr. Strand affirmed that dihydrotestosterone (DHT) and testosterone levels within tissues were measured using mass spectroscopy. He added that androgen levels within nodules were not captured and could not be used to rule out lack of drug penetration as a cause of glandular nodule resistance.

BREAKOUT SESSIONS: IDENTIFYING ROADBLOCKS AND PRIORITIES

Participants attended one of four breakout sessions. Guiding questions were provided to focus the discussions.

- **Group 1: Molecular and Cellular Etiology of BPH and LUTS**
  Moderators: *Teresa Liu, Ph.D., University of Wisconsin–Madison*
  Tracy Rankin, Ph.D., NIDDK, NIH
  Douglas Strand, Ph.D., The University of Texas Southwestern Medical Center
- **Group 2: Translational Approaches and Identification of New Therapeutic Targets**
  Moderators: *James Brooks, M.D., Stanford University*
  Ziya Kirkali, M.D., NIDDK, NIH
  Christopher Mullins, Ph.D., NIDDK, NIH

- **Group 3: Methods and Tools Needed to Interrogate BPH/LUTS Research Questions**
  Moderators: *Candace Kerr, Ph.D., NIA, NIH*
  William Ricke, Ph.D., University of Wisconsin–Madison
- **Group 4: Clinical Research Needs Approaches**
  Moderators: *Julie Barthold, M.D., NIDDK, NIH*
  Scott Bauer, M.D., UCSF and San Francisco Veterans Affairs Medical Center
  Claus Roehrborn, M.D., The University of Texas Southwestern Medical Center

INITIAL REPORTS FROM BREAKOUT SESSIONS
Moderator: Tracy Rankin, Ph.D., NIDDK, NIH

Dr. Rankin invited the breakout session leaders to report the results from their discussions, touching on the major points and themes.

Breakout Session 1

Dr. Strand reported on the group's discussions. A cellular definition of BPH has been enabled by identifying every cell type in normal and diseased prostates. Stromal nodules are composed of interstitial fibroblasts, and glandular nodules are composed of basal and luminal cells with some interstitial and peri-epithelial fibroblasts. The group agreed that a conceptual model for BPH progression has been difficult to develop. Fetal prostate development requires several known genes, but these genes are not overexpressed in BPH. Dr. Strand commented that it might be more productive to work backward from genes that are known to be upregulated in BPH. Mouse models will be critical for this work. The group agreed that no conclusive evidence exists for any single "first hit" required for the development of BPH, which is affected by a variety of molecular and physiological inputs. The group discussed reasons why BPH is restricted to the transition zone. It is possible that peri-urethral interstitial fibroblasts initiate BPH after receiving a localized insult from the urethra. Dr. Rankin added that the group discussed the role of inflammation as a potential driver of BPH.

Breakout Session 2

Dr. Brooks presented the group's deliberations. He noted the potential for new translational opportunities, including novel therapies and precision medicine approaches. Better subtyping of BPH is necessary to move forward. Such patient features as psychosocial issues, medication use, social determinants of health, body mass, and detailed symptoms should be recorded. DNA collection from patients will help in associating genetic variants with BPH subtypes. Anatomical and physiological characteristics—including the presence of intravesical projections and measures of bladder form and function—should be documented, and high-quality tissues and bioanalytes should be collected. All of these data should be compared to clinical endpoints, such as therapy failure. Dr. Brooks noted several challenges in the area of identifying new therapeutic targets, including the difficulty of collecting features related to the bladder and shortage of clinical samples for exploratory work. The group discussed strategies for obtaining healthy and BPH/LUTS tissues. Bladder samples are not collected during radical prostatectomies, and these patients are not well-characterized. Samples can be collected from patients undergoing needle biopsies for elevated PSA levels because many of these patients do not have cancer. Needle biopsies might be prone to sampling errors, however. The group noted that cystectomy patients and patients undergoing surgery following oral therapy failure could be sampled. The group discussed practical and ethical issues related to obtaining samples from paid volunteers.

Breakout Session 3

Dr. Ricke summarized the group's discussion. Current molecular imaging techniques are sufficient for BPH/LUTS researchers' needs. New computed tomography, MRI, elastography, and fibrotic tissue imaging techniques can be used to image various cell types. The group discussed animal models of BPH. Dogs have been the gold standard model for BPH research, but they are associated with ethical and ease-of-use challenges. New rat and mouse animal models have been developed in the past several years. Dr. Ricke emphasized the importance of linking physiology with cellular and molecular characteristics—using such techniques as scRNA-seq, spatial microscopy, chip assays, and organoid culture—to better understand the heterogeneity of BPH. The group agreed that more repositories and bioinformatic datasets are necessary. These resources will enable cross-referencing between human disease and experimental models. Dr. Ricke closed by mentioning novel biomedical engineering techniques that will enable high-throughput analysis of different cell types. He noted that R21 funding mechanisms could be useful in supporting the development of new technologies.

Breakout Session 4

Dr. Roehrborn summarized the group's deliberations. He noted that previous clinical studies ignored many factors (e.g., the role of inflammation, the role of prostate shape, detailed urodynamics, sleep, other parts of the LUT). The group agreed that study populations should be better characterized. New symptom scores should be evaluated with treatment trials. Detailed assessments of urodynamic measures (e.g., frequency volume charts, functional capacity, voiding efficiency, free flow rate) should be tied to clinical outcomes. Studies should engage with emerging technologies, such as point-of-contact ultrasounds and urometry analysis apps. Dr. Roehrborn listed novel biomarkers, endoscopy imaging, and bladder wall appearance as additional measures for better phenotyping of patients in trials and studies.

DAY 1 ADJOURNMENT
Tracy Rankin, Ph.D., NIDDK, NIH

Dr. Rankin explained the logistics for the following day and reviewed the remainder of the meeting agenda. The meeting was recessed at 4:10 p.m.

THURSDAY, MARCH 31, 2022

Recap of Day 1 and Plans for Day 2
Tracy Rankin, Ph.D., NIDDK, NIH

Dr. Rankin welcomed participants and reviewed the presentations and breakout discussions from Day 1. She highlighted such cross-cutting topics as the relationship between BPH and autoimmune diseases, the connection between fetal prostate development and BPH, the need for better and broader phenotyping of patients with BPH/LUTS, and the need for novel precision therapeutics.

TRAINEE SHORT TALKS AND POSTER SESSION

Participants viewed introductory videos prepared by poster presenters before joining breakout rooms to discuss individual posters.

1. **Prostate Immune Remodeling in Steroid Hormone Imbalance**
   Petra Popovics, Ph.D., University of Wisconsin–Madison
   Chronic inflammation in the prostate is associated with LUTS in aging men, but the connection between inflammation and steroid hormone imbalance is unclear. This study aimed to characterize early immunological changes driven by steroid hormone imbalance. Hormonal imbalance was generated by the surgical implantation of pellets containing steroid hormones in wild-type or immunocompromised osteopontin knockout (*Opn*-KO) mice. Steroid hormone treatment transiently increased immune cell infiltrations into the prostate, an effect that was diminished in immunocompromised mice. *Opn* expression levels were upregulated in infiltrating luminal macrophages harvested from wild-type steroid-treated mice; these cells were identified as foam cells. Infiltration by foam cells and increased *Opn* expression were associated with the early stages of prostate steroid hormone imbalance and as potential drivers of urinary dysfunction.

2. **MRI-Based Assessment of LUT Biomechanics During Voiding**
   Alejandro Roldan-Alzate, Ph.D., University of Wisconsin–Madison
   LUT biomechanics during voiding have been challenging to study because diagnostic tools are invasive and provide limited information. The goal of this study was to implement an MRI-based urodynamics protocol for the assessment of LUT biomechanics during voiding. One BPH patient and five healthy subjects were equipped with a condom catheter to void while being imaged in a 3T (Tesla) MRI scanner. Segmented volumes of the bladder were analyzed to calculate such measurements as bladder wall thickness, bladder volume, voided volume, flow rate, and post-void residual. Measurements were validated using uroflowmetry analysis. This novel, noninvasive, and comprehensive MRI protocol was implemented successfully to evaluate LUT anatomy, function, and biomechanics throughout the voiding cycle in a safe, accurate, and reproducible manner.

3. **Identification of Signature Genes/Pathways and Novel Therapeutic Strategies to Target Benign Prostatic Hyperplasia**
   Hamed Khedmatgozar, M.S., Texas Tech University Health Sciences Center
   BPH treatments are limited to 5ARIs or alpha-blocking drugs, both of which commonly fail. An urgent need exists to identify new molecular-based therapies for more effective management of BPH. To identify molecular targets, three human BPH RNA-seq datasets were integrated and common differentially expressed genes were identified across the datasets. Pathways associated with cell migration and endoplasmic reticulum membranes were enriched in samples from patients treated with 5ARI, and slit homolog 3 (*SLIT3*) was identified as a candidate gene for regulating BPH development. Reduction of *SLIT3* expression reduced proliferation in human epithelial and stromal BPH cells, and *SLIT3* knockdown impaired the ability of human BPH cells to form 3-D spheroids. *SLIT3* encodes a secreted signaling molecule that is a candidate regulator of BPH pathogenesis.

4. **TNF is a Potential Therapeutic Target to Suppress Prostatic Inflammation and Benign Prostatic Hyperplasia in Autoimmune Disease**
   Renee E. Vickman, Ph.D., NorthShore University HealthSystem
   Autoimmune diseases can affect many organs. The prostate, however, rarely is considered as a target organ of systemic inflammatory processes. This study utilized medical record data, patient samples, and *in vivo* models to evaluate the impact of inflammation on prostate tissue. Evaluation of 112,152 patient medical records indicated that BPH prevalence is significantly higher among patients with autoimmune diseases. Furthermore, treating these patients with TNF-antagonists

significantly decreases BPH incidence. Results from scRNA-seq and *in vitro* assays suggested that macrophage-derived TNF stimulates BPH-derived fibroblast proliferation. TNF blockade significantly reduced epithelial hyperplasia and ventral prostate volume in a mouse model of prostate enlargement. Additionally, TNF blockade significantly reduced prostatic epithelial hyperplasia, nuclear factor kappa B (*NFκB)* activation, and macrophage-mediated inflammation in prostate tissues from an autoimmune mouse model and human patients. Together, these studies show that patients with autoimmune diseases have a heightened susceptibility to BPH and that reducing inflammation with a therapeutic agent can suppress BPH.

5. **Aquablation Therapy: An Update on Minimally Invasive Therapy for BPH and Male LUTS**
   Tanmay Sarma, B.Pharm., Girijananda Chowdhury Institute of Pharmaceutical Science

   Innovative therapies for LUTS associated with BPH are urgently needed. Aquablation therapy is an effective and robust minimally invasive treatment option for LUTS in patients with BPH. Aquablation combines real-time multidimensional imaging, automated robotics, and heat-free waterjet ablation technology to remove prostate tissue—regardless of prostate structure or size—in a targeted and immediate manner with low risks of side effects. This procedure is equally as effective as TURP, with the added benefits of retaining sexual function and lowering patient morbidity and health care expenditures.

SESSION 3: PATHWAYS TO PATHOLOGY

Aging

Teresa Liu, Ph.D., University of Wisconsin–Madison

Dr. Teresa Liu discussed the role of aging in urinary tract biology. Aging includes normal processes (e.g., inflammation, cellular senescence, epigenetic alterations) but also can lead to pathologies (e.g., BPH/LUTS, cancer, diabetes) that can be halted by disease-specific preventions and treatments. Dr. Liu noted that delaying the effects of aging might be one approach to mitigate urinary dysfunction and disease. She pointed out that mice are a useful preclinical model for BPH. Prostatic urethral anatomy is similar in mice and humans, and aged mice spontaneously develop LUTD. LUTD in mice can be observed via increased fibrosis and proliferation in the prostate gland. Urinary dysfunction increases in these animals, as determined by analysis of urine spot assays with Void Whizzard imaging software. Because the use of aged mice often is impractical, treatment with steroid hormones can be leveraged to generate artificially aged mice. Prostate alterations in the mouse steroid hormone model of aging recapitulate multiple aspects of human disease.

Dr. Liu described several hallmarks of aging in the steroid hormone mouse model. Regarding epigenetic alterations, Dr. Liu explained that significant changes in steroid metabolism are observed in aged mice. These mice also exhibit hypermethylation of the promoter region of the cytochrome P450 family 7 subfamily B member 1 (*Cyp7b1*) gene. Loss of *Cyp7b1* expression is protective against urinary dysfunction in aged mice by increasing estradiol receptor β (ERβ) activation. With respect to cellular senescence, Dr. Liu described evidence of an increase in *p16*-positive cells in BPH tissues, indicative of increased senescence. The senescence accelerated mouse prone 6 (SAMP6) model was used to determine whether increased senescence can drive BPH. Following high-fat diet (HFD) feeding to induce metabolic syndrome, SAMP6 mice showed increased urinary dysfunction and fibrosis in the prostatic urethra compared to HFD-fed control mice. Lastly, increased mitochondrial dysfunction—as evidenced by increased expression of the mitochondrial respiratory complex I (Complex I) subunit, NADH:ubiquinone oxidoreductase core subunit S3 *(NDUFS3)*—has been observed in BPH tissues. Chemical inhibition of Complex I by rotenone in human stromal prostate cells results in increased expression of genes associated with fibrosis, mitochondrial function, and senescence.

Dr. Liu concluded that aging biomarkers must be developed for better disease stratification and treatment of BPH/LUTS. Metabolomic analysis of blood and urine can be used to identify alterations in mitochondrial dysfunction, quantify senescence-associated secretory phenotypes, and determine epigenetic ages of patients. Physiological changes associated with aging, such as insulin resistance and increased frailty, also should be investigated.

Discussion

- Dr. Rankin asked whether future studies would focus on stromal or glandular cells. Dr. Liu answered that her group will investigate both epithelial and stromal cells, as well as crosstalk between the two groups. In response to a question from Dr. Rankin about where the *p16* senescence marker was expressed, Dr. Liu explained that *p16* expression was observed in both stromal and glandular cells. Dr. Rankin wondered whether secretion was altered in senescent cells.
- Dr. Bauer asked about experimental paradigms for exercise in mice. Dr. Liu noted that running wheels commonly are used.
- In response to a question from Dr. Aria Olumi (Beth Israel Deaconess Medical Center) about the effects of DNA methylation on steroid levels and prostate function and size, Dr. Liu answered that mass spectrometry experiments are underway to measure hormone levels in tissues from *Cyp7b1* knockout mice.

Immune Dysfunction
Travis Jerde, Ph.D., Indiana University School of Medicine

Dr. Travis Jerde provided an overview of inflammation and BPH. Dr. Jerde described detailed characteristics of acute and chronic inflammation. He noted that recalcitrant inflammation—inflammation that has lost its self-limiting capabilities—can last indefinitely and is characterized by fibrosis and necrosis, massive tissue remodeling, mixed acute and chronic cellular infiltrates, and a unique array of inflammatory mediators (e.g., interleukin 6 [IL-6], IL-8, developmental morphogens). Dr. Jerde pointed out that prostatic inflammation can be bacterial in origin but most often is a form of dysfunctional inflammation that is similar to recalcitrant inflammation. Dr. Jerde highlighted that dysfunctional inflammation differs from recalcitrant inflammation in that it is triggered by reinitiating internal signals rather than persistent external signals. He commented on the lack of cellular or molecular markers to differentiate between normal and dysfunctional inflammation.

Dr. Jerde discussed proposed causes of prostatic inflammation, including PSA and prostate alkaline phosphatase. He pointed out that PSA is included as an adjuvant in PROSTVAC immunotherapy to activate the immune response. Rectal bacteria and associated antigens also might be a source of inflammation in the prostate. Dr. Jerde highlighted an experiment published in 1982 showing that activated charcoal in men's bladders refluxed into the prostate during BPH, representing another possible route for inflammatory triggers. Dr. Jerde listed several immune cell types (e.g., T cells, B cells, dendritic cells, M1 and M2 macrophages, neutrophils), signals, and antibody classes that have been associated with histological BPH. He noted that these data are consistent with both recalcitrant inflammation and immune dysfunction. Dr. Jerde listed current pharmacological therapies for BPH, many of which target inflammation or inflammatory signaling. He concluded with several models of inflammation—including the induction of BPH-like nodules with *Toxoplasma gondii* parasites or bisphenol A—to investigate the central relationship between immune responses and prostatic inflammation in BPH.

Discussion

- Dr. Rankin asked about additional evidence for increased urinary reflux into the prostate in aging men. Dr. Jerde responded that he has not seen any other literature regarding this phenomenon.

Androgen Signaling Pathways
Aria Olumi, Ph.D., Beth Israel Deaconess Medical Center

Dr. Aira Olumi discussed signaling pathways related to BPH. His research group is interested in defining mechanisms of resistance to 5ARIs, one of the main forms of treatment for BPH. Studies have shown that symptom progression is slowed in only 34 percent of patients with BPH treated with 5ARIs. These medications act on the steroid 5 alpha-reductase 2 (SRD5A2) protein, which converts testosterone to DHT and is the most prominent 5 alpha reductase found in the prostate. Dr. Olumi noted that the *SRD5A2* promoter can be methylated, a process associated with reduced gene expression. Around one-third of adult men do not express *SRD5A2* in their prostates. He added that inflammatory mediators regulate *SRD5A2* promoter methylation and expression via the TNFα pathway. In the absence of *SRD5A2* expression, an androgenic to estrogenic switch is initiated, and testosterone is converted to estradiol, a less potent androgen. Dr. Olumi pointed out that this pathway is an opportunity for novel and targeted therapies.

Estrogen signaling in the prostate has been well-studied in the context of development and growth. Dr. Olumi's group observed increased estradiol levels in prostates of patients with low *SRD5A2* expression and hypothesized that non-androgenic pathways are activated to maintain prostatic development and growth when *SRD5A2* expression is suppressed. Novel *Srd5a2* targeted deletion mice were generated. Urogenital organs, including the prostate, develop but are significantly smaller in *Srd5a2* knockout mice. Expression analysis using scRNA-seq showed that luminal epithelial cells are altered in the knockout mouse prostates. Although overall levels of luminal epithelial cells are reduced, significantly increased levels of a single luminal epithelial cell population (termed LE2) were observed. Further analysis showed that the LE2 gene signature—which is associated with expression of the *estrogen receptor 1* (*Esr1*) gene—is localized to the anterior prostate lobe. LE2 cells in *Srd5a2* targeted deletion mice also show increased stromal input via the WNT family member 5A (*Wnt5a*) pathway, which has been shown to inhibit epithelial stem cell activity via transforming growth factor β (TGFβ) signaling. Dr. Olumi proposed that altered WNT/TGFβ signaling in *Srd5a2* knockout mice leads to increased survival and growth of the LE2 cell population, independent of the presence of androgens. He noted that *WNT5a* expression levels correlate with *SRD5A2* expression in human prostate samples.

Discussion

- In response to a question from Dr. Strand regarding the heterogeneity of *SRD5A2* expression across the prostate, Dr. Olumi explained that a previous investigation of *SRD5A2* expression found 90 percent concordance of expression or loss of expression across three different regions of the prostate.
- Dr. Vezina commented that the *Srd5a2* targeted deletion mice were generated in Dr. Andy McMahon's laboratory with support from the GenitoUrinary Development Molecular Anatomy Project (commonly known as GUDMAP). He noted that, despite low green fluorescent protein expression levels, the Cre recombinase is effective in these mice.

Fibrosis and LUTS
William Ricke, Ph.D., University of Wisconsin–Madison

Dr. William Ricke highlighted the lack of new BPH therapies in the past two decades and noted that BPH remains a deadly disease in most of the world. He listed such underlying causes of urinary obstruction as urethral obstruction by BPH nodules, deficiencies in urethral smooth muscle contractility, and fibrosis in the area surrounding the urethra. Dr. Ricke presented an MRI of an 8-pound prostate in a patient with minimal symptoms and emphasized that prostate size and urinary symptoms do not necessarily overlap. He noted that alpha-blocking drugs and 5ARIs are prescribed to treat smooth muscle failure and hyperplasia, respectively, but that fibrosis has not yet been leveraged as a clinical target.

Dr. Ricke defined fibrosis as the deposition of connective tissue that can alter or destroy the architecture and function of the associated organ. He pointed out increasing evidence in the literature for a connection between fibrosis and both human and experimental models of LUTD. Fibrosis and collagen genes are upregulated in LUTD transcriptional datasets, and collagen deposition in the human prostate correlates with men's age and BPH progression. Dr. Ricke added that numerous animal models of BPH (e.g., SAMP6+HFD mice, aged mice, steroid-treated mice, mouse prostatitis models, dogs) also exhibit prostatic fibrosis. Preclinical assessments of anti-fibrotic drugs have been promising. Treatment of prostate stromal cells with thalidomide reduces expression of collagen genes, and treatment of aged mice with halofuginone normalizes voiding behaviors. Dr. Ricke pointed out that fibrosis is known to occur most commonly in peri-urethral and inter-nodule regions, but its presence in other anatomical locations and its associated cell types and molecular mechanisms still must be delineated. He described efforts by his group to identify urinary biomarkers of fibrosis in humans and mice via mass spectrometry.

Discussion

- In response to a question from Dr. Smita De (Cleveland Clinic) regarding mouse models of BPH, Dr. Ricke affirmed that mouse bladders develop trabeculations.
- Dr. Kristina Penniston (CAIRIBU Interactions Core) asked whether prostatic fibrosis correlated with systemic fibrotic processes. Dr. Ricke answered that he does not have a conclusive answer yet, but that experiments are underway to address this question.
- Dr. Goueli wondered whether Dr. Ricke's group had evidence that changes in bladder fibrosis were contributing voiding behaviors in halofuginone-treated mice. Dr. Ricke responded that his group has collected bladders from these mice but have not yet analyzed these samples. He acknowledged the need to be mindful of the many physiological aspects contributing to voiding in this model.
- Dr. Praveen Thumbikat (Northwestern University) wondered whether anti-fibrotic treatments were reverting or halting fibrosis and asked whether Dr. Ricke's group uses biomarkers to track the resolution of fibrosis. Dr. Ricke answered that his group currently is using molecular and immunostaining techniques to monitor the presence of fibrosis, and those results will be forthcoming. His group has observed resolution of fibrosis using MRI techniques.
- In response to a question from Dr. Olumi about differential prostate growth patterns across patients, Dr. Ricke pointed out that the environment likely plays an important role.
- Dr. Matusik commented that the plasminogen-activator pathway, which is activated in BPH and known to cause fibrosis in the heart, has been overlooked and should be investigated. He added that blocking the plasminogen-activator pathways in organ explant cultures of newborn mouse prostates resulted in inhibition of prostate budding and branching. Dr. Ricke agreed that

extracellular matrix (ECM) mediators likely play a role in BPH. He pointed out that—unlike 5ARIs, which take months to exert effects—anti-fibrotic drugs appear to act fairly quickly.

- Dr. Bauer asked whether protective factors should be investigated in patients with enlarged prostates but minimal symptoms. Dr. Ricke responded that this would be a highly fruitful area to investigate. Participants agreed that stratifying patients based on both objective and subjective measures of BPH is necessary.
- Dr. De commented on the necessity to investigate biomechanical properties of different forms of BPH (e.g., glandular vs. stromal).

SESSION 4: RELATIONSHIP BETWEEN PATHOLOGY AND SYMPTOMS

Role of Bladder–Prostate Crosstalk: Neural Manifestations

Janet Keast, Ph.D., University of Melbourne

Dr. Janet Keast provided an overview of neuronal regulation of the urological system. Motor neurons are autonomic, two-neuron pathways defined as sympathetic and parasympathetic based on their level of spinal control. They carry motor signals from the central nervous system (CNS) to the prostate or bladder. Parasympathetic motor neurons regulate detrusor contraction, urethral relaxation, and prostate gland secretion. Sympathetic motor neurons regulate smooth contraction in the bladder neck, urethra, and prostate, as well as arterial vessel constriction. Sensory neurons detect changes in the organ's microenvironment (via mechanoreceptors and nociceptors) and convey this information to the CNS to generate an appropriate response. Dr. Keast pointed out that LUT neurons with different functions are intertwined, making functional separations challenging. She also noted that nerves can be regulated by non-neural cell types (e.g., immune cells, neuromodulator cells, interstitial cells, myofibroblasts, glia). Dr. Keast highlighted the difficulties of imaging the 3-D structure of neurons and understanding the nerve–organ interface. She emphasized the need to understand the types and locations of neuronal structures, as well as different methods of tissue preparation prior to analysis. She highlighted areas of opportunity in the field, including taking advantage of transcriptomic datasets to identify specific neural subclasses affected by perturbation and identify neural targets for stromal, epithelial, and muscle-derived factors.

Dr. Keast discussed crosstalk between the bladder and prostate. An altered prostate microenvironment can result in changes to prostate innervation, and obstruction can alter LUT mechanics, microvasculature, fibrosis, and inflammation. She noted that she has been unable to find a detailed study of altered neural patterning in BPH. Dr. Keast pointed out that, historically, neural circuits of the prostate and bladder have been considered separate units. She presented recent evidence for more direct communication between the two organ systems. For example, evidence exists that afferent axons in these systems bifurcate and innervate both the bladder and the prostate. It is possible that efferent axons bifurcate to both organs as well. Neuronal circuits from the bladder and prostate also might converge in the pelvic ganglia or the dorsal horn of the spinal cord. Dr. Keast emphasized that identifying neural causes of and contributors to early disease might aid in the understanding of BPH progression. She highlighted several examples of persistent changes to neural functions independent of the magnitude or recovery of the initial challenge (e.g., endometriosis pain, neuropathic pain, interstitial cystitis/bladder pain syndrome) and noted that neural contributions to prostate-independent LUTS—where prostate size and the presence of obstruction do not correlate with symptoms—should be assessed. Dr. Keast commented that newer techniques, such as optogenetics, can be used to selectively perturb bladder or neural pathways for these investigations.

Discussion

- Dr. Rankin asked whether neural cell changes associated with BPH have been observed in transcriptomic data. Dr. Keast noted that long axonal processes do not contain RNA; any transcriptional changes would take place within neuronal cell bodies, which are found within ganglia located distantly from the LUT.
- Dr. Strand asked whether ECM alterations in diseased organs could drive sensory neuron changes and cause differences in pain. Dr. Keast answered that evidence shows that axon terminals can expand in respond to several negative stimuli. She added that altered sensory behavior resulting from changes in neuronal structure is an appealing hypothesis.
- In response to a question from Dr. Thumbikat about whether irritative symptoms might be driven primarily by neural sensitization, Dr. Keast agreed that this is likely. Studies in animals with perturbed prostates or bladders have shown increasing prostate innervation leading to increased sensitivity. These neurons have been shown to express the transient receptor potential cation channel subfamily V member 1 gene, a classic pain-sensing receptor. Dr. Keast pointed out that effects of alterations in mechanoreceptors, which are not visible using current markers, might be connected to non-pain symptoms. Dr. Thumbikat asked about assessing roles of sensory components using ablation. Dr. Keast answered that optogenetics can be used to selectively ablate or activate sensory neurons.
- Dr. Bauer noted that older patients with chronic pain at multiple sites were at an increased risk for LUTS and LUTS progression. He asked for more information about how pain parameters change with aging. Dr. Keast answered that the aging question was important. Her studies in the field of complex pain have shown that complex behavioral background, although challenging to study, is critical in the study of organ maladaptation.
- In response to a question from Dr. Ricke about insight from comparative analysis of bladders across species, Dr. Keast responded that several species have interesting bladder functions. The amphibian cloaca is a common chamber for the urinary tract, reproductive tract, and alimentary canal. Amphibian sphincter musculature also varies distinctly from mammals. Dr. Keast noted that she is more familiar with species-based differences in the vas deferens—which is similar to prostate smooth muscle—where an inverse relationship exists between the density of sympathetic innervation and spontaneous non-urination smooth muscle activity.

Pathways Forward to Understanding Bladder Dysfunction Post-BPH/Adaptations of Bladder
Ramy Goueli, M.D., The University of Texas Southwestern Medical Center

Dr. Ramy Goueli described pathways to understanding bladder dysfunction associated with BPH. He provided an overview of the bladder as a storage and evacuation organ. Dr. Goueli reviewed central and peripheral neural control of the bladder and its modulation by several medications. BOO can be caused by urethral strictures, congenital malformations, malignancy, functional obstruction, and BPH/BPE. Consequences of BOO include detrusor hypertrophy, followed by detrusor compensation (increased detrusor contractility often seen with detrusor overactivity as a result of prolonged high pressure) and, ultimately, detrusor decompensation (characterized by detrusor underactivity). The time between the onset of compensation and decompensation is variable and dependent on several factors (e.g., age, severity, type, comorbidities).

Dr. Goueli described the pathophysiology of LUTS resulting from BOO. On obstruction, the process of ischemia and reperfusion leads to increased oxidative stress in the bladder wall. Hypoxia upregulates the hypoxia-inducible factor and vascular endothelial growth factor pathways. Smooth muscle cell hypertrophy and an initial deposition of ECM characterize this early phase. As the adaptive responses

reach their limit, the compensation phase initiates and is characterized by continued ECM deposition. The final decompensation phase is characterized by urothelial dysfunction, neuronal and smooth muscle cell degeneration, and fibrosis. Dr. Goueli described the challenge of using animals to model obstruction, which results in acute BOO but might not replicate chronic disease in humans.

Dr. Goueli reviewed the various stages of detrusor dysfunction and wondered about the reversibility of this pathology. In a 10-year follow-up study of patients with untreated BOO, patients exhibited significant decreases in bladder contractility and significant increases in detrusor overactivity. Dr. Goueli described technology-based difference in LUTS after surgery, noting no differences between monopolar and bipolar TURPs and no differences between laser vaporization when compared with monopolar TURP after 24 months. Dr. Goueli pointed out that up to one-half of patients will experience persistent LUTS following outlet procedures. He noted that improvements in patient storage symptoms following outlet procedures are slower and more moderate than improvements in voiding symptoms.

Dr. Goueli defined OAB symptoms as urinary urgency, usually accompanied by frequency and nocturia, with or without urgency incontinence in the absence of urinary tract infection or other obvious pathology. The prevalence of OAB is reported as being between 12 and 30 percent and is greater in women than in men. OAB symptoms increase in women in their 40s and men in their 50s and 60s. The health burden of OAB is multifactorial, with sleep, self-esteem, sexuality, and overall health being affected. More than 90 percent of patients fail initial treatments for OAB within 2 years; most patients discontinue treatment within the first 6 months. The total cost of OAB in the United States is estimated to be almost $90 billion annually. Dr. Goueli described the treatment paradigm of OAB, which begins with behavioral treatments, followed by pharmacological management with beta 3 receptor agonists and anticholinergics, followed by third- and fourth-line surgical therapies.

Dr. Goueli returned to a discussion of bladder decompensation, the most devastating result of BOO. Detrusor underactivity due to decompensation affects up to 50 percent of men older than 70. Several causes have been proposed for increased detrusor underactivity, including age, diabetes, neurological disorders, dysfunctional voiding, and prolonged BOO. The outcome data of men with detrusor underactivity following outlet procedures is mixed, and treatment options for this condition are extremely limited. Dr. Goueli described the approach to patients with LUTS at his institute. He pointed out that prostate and bladder samples are collected for multi-omic analysis from patients undergoing prostatectomies. Future studies will include the use of artificial intelligence in urodynamic analysis, the correlation of bladder wall thickening and degree of BOO, real-time voiding MRI in the assessment of voiding function, and the use of multi-omic analysis to decipher the molecular pathways involved in bladder remodeling and parameters for recovery of bladder function.

Discussion

- Dr. Rankin asked about bladder dysfunction in men with larger prostates but no obstruction. Dr. Goueli noted that obstruction that is undetectable in the clinic still might be able to cause bladder dysfunction. Phenotyping patients with bladder dysfunction is critical. He wondered whether asymptomatic patients exhibiting voiding perturbations by MRI would eventually suffer from detrusor pathologies.
- In response to a question from Dr. Brooks about obtaining bladder samples, Dr. Goueli noted that samples were taken from the bladder dome. He has performed almost 20 of these procedures and patients have not experienced complications. Dr. Roehrborn commented that he has performed laser enucleation of the prostate on more than 100 patients. Prostate samples are collected and placed on ice within 30 minutes of the procedure, and specimens contain no coagulation artifacts.

A tissue segment that contains the full thickness of the bladder wall also is taken (and freshly measured) from most patients.

- Dr. Brooks noted a recent increase in failed surgical procedures. He wondered whether the use of oral treatments was postponing surgical interventions required to prevent bladder damage. Dr. Roehrborn commented that further research is necessary to determine whether surgical interventions actually clear obstructions. He mentioned evidence that waiting has a negative effect on outcomes, including a study showing that patients were far less likely to have successful outcomes if they were monitored with watchful waiting before undergoing surgical procedures than if they underwent surgery immediately after diagnosis. Dr. Goueli emphasized the importance of correctly phenotyping patients before suggesting treatments. For example, patients obstructed at the pelvic level will not experience relief following such prostate procedures as TURPs. Early interventions will be successful only if they address underlying pathologies.
- Dr. Bauer noted the lack of prospective studies of deeply phenotyped patients, because studies that begin with symptomatic patients likely are subject to many biases. Dr. Goueli suggested performing urinary assessments of patients in their mid-40s, similar to colonoscopies that are performed when patients turn 50. Participants discussed building a catalogue of bladder images from younger male and aged-matched female patients to compare with bladder images from men with BPH.
- Dr. Brooks commented that his experience with 3-D imaging of patients has shown him that the bladder outlet varies extensively from patient to patient to accommodate obstructions. He asked whether analysis of these changes could provide insight into the disease. Dr. Goueli answered that more information about altered voiding parameters would be useful for prescribing tailored medications. He does not prescribe cholinergic drugs, which increase bladder muscle contractions, to patients unless he has evidence that they are not obstructed.

DAY 2 ADJOURNMENT
Tracy Rankin, Ph.D., NIDDK, NIH

Dr. Rankin thanked the presenters and participants for the rich discussions. The meeting was recessed at 3:59 p.m.

FRIDAY, APRIL 1, 2022

Plans for Day 3 and Announcements
Tracy Rankin, Ph.D., NIDDK, NIH
William Ricke, Ph.D., University of Wisconsin–Madison

Dr. Rankin welcomed participants and introduced the last scientific session on tools. She explained that extra time was built into the breakout sessions, which will take place later in the day, to compile the results of the sessions.

Dr. Ricke announced that the Society for Basic Urologic Research will be hosting its spring meeting on Saturday, May 14, at the Hilton New Orleans Riverside. The theme will be aging of the lower urinary tract and hormone resistance mechanisms.

The University of Wisconsin–Madison O'Brien Center Symposium will take place June 29–30. The theme will be targeting aging in the lower urinary tract, and the keynote speaker will be Dr. Judith Campisi.

A follow-up meeting to the present workshop will take place at the UW–Madison Health Sciences Learning Center from 1:00–5:00 p.m. CDT on June 30. The goal of the follow-up meeting is to develop research and proposal ideas and generate a white paper based on the present workshop.

Dr. Rankin invited participants to join CAIRIBU efforts and activities.

SESSION 5: APPROACHES, TECHNOLOGIES, DATA SOURCES, AND TOOLS

High-Throughput Interrogations

James Brooks, M.D., Stanford University

Dr. Brooks discussed high-throughput approaches to understanding BPH. His group focuses on transcriptomic analysis of bulk human tissues to provide a broad view of BPH. These efforts are supplemented with other technologies to provide a more in-depth view. Dr. Brooks noted that different approaches to analyzing gene expression data can provide varying insights. Unsupervised analysis of prostate tissue transcriptomes can show BPH subtype groupings that are distinct from normal tissue. He provided the example of unsupervised hierarchical clustering being used to differentiate between more and less stromal classes of BPH—a difference that could not be detected by pathology. The stromal transcription signature correlates with clinical outcomes, such as symptom score and bothersome index. This technique can be used to investigate hypotheses (e.g., the correlation between fibrosis and LUTS progression) and identify new subtypes of BPH.

Dr. Brooks explained that supervised high-throughput approaches can be used to investigate candidate genes. He presented data demonstrating that candidate bioactive ligand genes (e.g., bone morphogenic protein 5 [*BMP5*], C-X-C motif chemokine ligand 13 [*CXCL13*]) are upregulated in BPH; cell-type specific markers (e.g., chromogranin [*CHGA*], *ESR1*) are downregulated. Gene expression analysis also can be used as an endpoint in *in vitro* models to show that patterns resemble human disease. When cultured prostate epithelial cells are treated with BMP5, a BPH-like gene expression signature is detected. Dr. Brooks pointed out that scRNA-seq can be used to identify expression patterns within individual BPH cell types. He noted that upregulation of *BMP5* and *CXCL13* specifically is observed in a subset of BPH fibroblasts. These data can be used to generate cell interaction networks from candidate ligand-receptor pairs. Dr. Brooks emphasized that scRNA-seq is a challenging and expensive technique that can yield variable results and make quantitative comparisons across BPH cases difficult. Imputed interactions must be validated. By its very nature, scRNA-seq loses spatial relationships between cells and poorly represents small cell populations. Dr. Brooks listed several new multiplex immunohistochemistry techniques to overcome these deficiencies, including CO-Detection by indEXing (commonly known as CODEX), multiplexed ion beam imaging by time-of-flight (commonly known as MIBI-TOF), and Cell DIVE™. These techniques can image up to 100 antibodies simultaneously but require single cell segmentation software and sophisticated analytical approaches. Dr. Brooks expects that robust multiplex immunohistochemistry pipelines will be established during the next few years.

Dr. Brooks listed several high-throughput genomic approaches. Evidence suggests that DNA methylation patterns are altered in BPH tissues. Assay for transposase-accessible chromatin using sequencing (commonly known as ATAC-seq) can be used to analyze chromatin structure. The relationships between BPH and many biological features (e.g., non-coding RNAs, RNA methylation, DNA-protein interactions) have yet to be explored. Sequencing can be used to track clonal cell population dynamics by tracking the origin and expansion of single nucleotide variants (SNVs). New metabolomics approaches, such as desorption electrospray ionization mass spectrometry imaging (DESI-MSI), can be used study metabolites across histological tissue sections. He provided DESI-MSI results showing a distinct cutoff at the border between normal and malignant prostate tissues, as determined by an increased glucose-to-citrate ratio. This technique has been able to detect metabolomic alterations in the kidney after obstruction, including

changes to hydrophobic metabolites that are not detectable in urine. Mass spectrometry wands can be used in real-time in operating rooms. Proteomic analysis can circumvent challenges associated with post-transcriptional regulation, and improved methods have enabled proteomic analysis of even small amounts of tissue. Single cell proteomics can be performed on disaggregated tissues. Dr. Brooks discussed glycoproteomic approaches and explained how alterations in glycosylation can be observed when comparing healthy and cancerous tissues. Cancerous prostate cells exhibit increased sialylation, a potential mechanism for evading immune detection. Dr. Brooks summarized the need to explore BPH biology using diverse high-throughput approaches.

Discussion

- Dr. Strand commented on discrepancies between the transcriptome and proteome and noted that the BPH field should shift its focus to the proteome. He asked whether bulk proteomics on BPH tissues could be deconvoluted into cell-type specific information. Dr. Brooks answered that this could be performed crudely using cell lineage markers. He agreed that proteomics should be prioritized.
- A participant noted that such methods as matrix-assisted laser desorption/ionization could improve DESI-MS resolution. Dr. Brooks agreed that DESI-MS can be approved. He added that the resolution of DESI-MS was sufficient for his group's intention to identify relevant metabolites quickly in particular tissue types.
- In response to a question from Dr. Ricke regarding the loss of biological information during tissue disaggregation, Dr. Brooks commented that information gets lost, but the resulting data can still be informative.
- Dr. Star asked about quality-control measures before sample analysis. Dr. Brooks described histological and RNA library quality checks and noted that analytical pipelines must be annotated carefully and made available in the public domain. He pointed out that unsupervised clustering is an excellent unbiased method for quality control; if samples do not cluster into accurate pools (e.g., control vs. BPH, healthy vs. cancerous), they are unlikely to yield quality data.

Novel Imaging Approaches
Peter Caravan, Ph.D., Massachusetts General Hospital

Dr. Peter Caravan discussed molecular approaches to imaging fibrosis, a dysregulated tissue response associated with such injuries as chronic inflammation that leads to excessive accumulation of ECM components and organ dysfunction and failure. Noninvasive imaging of fibrosis is a major unmet need that would enable the measurement of disease progression and treatment response. Dr. Caravan explained that collagen-targeted MRI contrast agents are being leveraged for molecular imaging of fibrosis. He presented data showing that a cyclic peptide probe containing a gadolinium (Gd) moiety can be used to image fibrotic burden associated with liver steatosis. Imaging readouts are quantitative and correlate with biochemical collagen markers. Dr. Caravan described how fibrosis imaging was used to track disease progression and response to treatment in a rat model of bile duct ligation. He added that 3-D MRI could be used to visualize partial and heterogenous responses to treatment within a single organ.

Dr. Caravan described his group's efforts to image the active process of fibrotic tissue formation. He pointed out that oxidized lysines (aldehydes [Hyd] named allysines) are a byproduct of lysyl oxidase activity during fibrogenesis. The presence of allysines is temporary because they eventually undergo condensation reactions to form stable collagen crosslinks. Dr. Caravan explained that a Gd-Hyd probe was developed to detect oxidized collagen as a tractable marker of active fibrogenesis. This contrast agent is hydrophilic, stable, and target-selective, with rapid blood clearance through renal elimination. Next-

generation probe design was used to generate dual-binding probes with improved binding and signal strength. In a mouse model of liver fibrosis, MRI signal from dual probes enabled early detection of disease and early response to treatment. MRI signal also correlated with histological markers of fibrogenesis but not total collagen burden. The dual probes have been validated in a rat model of biliary stasis and human tissues samples from hepatic steatosis patients. Dr. Caravan noted that collagen and fibrogenesis imaging have been used in multiple organ systems and can be modified for functional positron emission tomography (PET) in human patients.

Dr. Caravan closed with a discussion of other noninvasive imaging tools for use in the prostate. A Gd-CP027 probe was developed to image zinc (Zn) in the prostate and validated in mice and human patients. Manganese can be used to probe calcium flux. Reactive oxygen species can be monitored using a redox-active iron complex, Fe-PyC3A, as an MRI contrast agent.

Discussion

- Dr. Ricke asked whether a Zn tracer could detect BPH noninvasively. Dr. Caravan answered that Zn imaging would be more informative if its function in BPH was known. Dr. Ricke asked about merging imaging modalities (i.e., Zn and collagen) for more meaningful results. Dr. Caravan stated that this would be feasible because the probes are cleared very quickly. Multiple probes have been combined to assess livers in animals.
- Dr. Olumi asked about radiological imaging and measuring of fibrotic and inflammatory effects simultaneously. Dr. Caravan answered that it was possible to combine collagen imaging with fluorodeoxyglucose-enhanced PET or ferumoxytol-enhanced MRI to visualize inflammation.
- Dr. Brooks asked about easing the approval process for patient use of these tools. Dr. Caravan pointed out that the small reagent volumes used for PET techniques preclude the need for extensive preclinical safety and toxicology studies. PET has several downsides, however, including lower image resolution and operational challenges associated with manufacturing fresh reagent the day of imaging. Dr. Caravan commented that MRI techniques must undergo the same costly and labor-intensive approval process as therapeutics. He thanked the NIDDK and National Cancer Institute for their assistance with the approval of one of his group's imaging compounds.

Utilizing Electronic Health Record (EHR) Data to Understand Disease
Tina Hernandez-Boussard, Ph.D., Stanford University

Dr. Hernandez-Boussard reported on integrating and analyzing EHR data to better understand BPH and LUTS. The utility of EHR data became apparent after Congress passed the 21st Century Cures Act, which requires regulators to use diverse types of evidence (outside of RCTs) to approve new drugs and devices. EHR data are accessible and detailed but also challenging to analyze. Information recorded in EHRs is not intended for research use, documentation reliability is variable, and interoperability between different EHR systems is limited. Dr. Hernandez-Boussard described the use of EHR data for clinical trial applications. Artificial intelligence can be used to identify candidate patients for clinical trials based on desired medical characteristics and the patients' likelihoods of completing trials. She provided an example of identifying patients using continuous glucose monitoring of type 1 diabetes to compare with a control group that was using alternate methods (e.g., finger-stick monitoring). These data were not captured explicitly in EHRs but could be gathered reliably using a natural language processing (NLP) algorithm.

Participants were updated on the efforts to identify patient-centered outcomes (i.e., net effects of a disease or disease treatment), which are not captured systematically and are not integrated into the medical record as structured data. Dr. Hernandez-Boussard presented her group's workflow for assessing EHR unstructured text—which includes text pre-processing, term recognition, contextual processing, and

knowledge extraction stages—to develop a matrix of clinical terms that can be used for further analysis. She described several algorithms used in data transformation pipelines to extract functional data or patient-centered outcomes. Dr. Hernandez-Boussard compared the ability of EHR structured and unstructured data to capture various medical conditions. EHR structured data can capture such events as surgeries accurately (likely because of international classification of diseases [ICD] codes used for health insurance billing); incidences of diabetes, however, are captured more accurately by unstructured data. She described results from the Comparative Effectiveness Analysis of Surgery and Radiation study—published in the *Journal of the American Medical Association*—investigating the documentation of urinary incontinence (UI) following prostatectomy using ICD codes compared with unstructured data. Whereas four positive cases of UI and zero negative cases (out of 5,353 EHRs) were documented by ICD codes, a rule-based NLP identified 450 positive cases of UI and confirmed 1,035 negative cases.

Extraction of regulatory-grade data from EHRs in the absence of explicit guidelines is a challenge. Dr. Hernandez-Boussard described her efforts to investigate the representativeness of RCT data compared with real-world data. Data from four RCTs was used to build a prognostic model for metastatic castration-resistant prostate cancer patients. Dr. Hernandez-Boussard's group compared demographic information collected from this RCT data with EHR data and noted that EHR data contained more information from a wider range of ages and races. Although the RCT data required 101 variables for model optimization, EHR data could be trained to generate an accurate model framework using just 10 variables. Dr. Hernandez-Boussard closed with a description of her "digital twin" model for patient data, in which genomic, molecular, imaging, and clinical data are pooled together to train models to simulate patient outcomes before making individualized patient care decisions.

Discussion

- Dr. Roehrborn commented on the need for accurate documentation in EHRs. He added that clinicians tend to copy and paste material from one EHR to another, resulting in loss of detailed, patient-specific information. Dr. Hernandez-Boussard agreed that this is a challenge but noted that sophisticated algorithms are being developed to deal with this issue.
- Dr. Bauer reflected that the BPH/LUTS field is plagued by extremely rare but serious outcomes associated with surrogate markers for BPH. He asked for more information about using EHR data to stratify patient risk and build outcome prediction models. Dr. Hernandez-Boussard answered that more accurate models will be developed as more cases become available to train models. Her group has been able to use EHR data mining to predict outcomes of spinal fluid leaks, which are similarly confounding.

BREAKOUT SESSIONS: IDENTIFYING ROADBLOCKS AND PRIORITIES

Participants attended one of four breakout sessions. Guiding questions were provided to focus the discussions.

- **Group 1: Molecular and Cellular Etiology of BPH and LUTS**
  Moderators: *Teresa Liu, Ph.D., University of Wisconsin–Madison*
  Tracy Rankin, Ph.D., NIDDK, NIH
  Douglas Strand, Ph.D., The University of Texas Southwestern Medical Center
- **Group 2: Translational Approaches and Identification of New Therapeutic Targets**
  Moderators: *James Brooks, M.D., Stanford University*
  Ziya Kirkali, M.D., NIDDK, NIH
  Christopher Mullins, Ph.D., NIDDK, NIH

- **Group 3: Methods and Tools Needed to Interrogate BPH/LUTS Research Questions**
  Moderators: *Candace Kerr, Ph.D., NIA, NIH*
  William Ricke, Ph.D., University of Wisconsin–Madison
- **Group 4: Clinical Research Needs Approaches**
  Moderators: *Julie Barthold, M.D., NIDDK, NIH*
  Scott Bauer, M.D., UCSF and San Francisco Veterans Affairs Medical Center
  Claus Roehrborn, M.D., The University of Texas Southwestern Medical Center

FINAL BREAKOUT SESSION REPORTS AND GROUP DISCUSSION
Moderator: Tracy Rankin, Ph.D., NIDDK, NIH

Dr. Rankin invited breakout session leaders to report the final results from their discussions.

Breakout Session 1

Cellular definition of BPH. Dr. Strand reported the group's cellular and anatomical definitions of BPH. BPH tissue is a heterogeneous composition of peri-urethral stromal and glandular phenotypes that are unique to each patient. Stromal phenotypes consist of expanded interstitial fibroblasts, smooth muscle, and autoimmune infiltrates. Glandular phenotypes are composed of prostate epithelia, peri-epithelial fibroblasts, interstitial fibroblasts, and autoimmune infiltrates. BPH is characterized by an increase in blood vessel growth and possible expansion of neural networks.

Conceptual model of branching morphogenesis in prostate development and disease. Dr. Strand described a framework for initiation in which branching morphogenesis is activated by reawakened interstitial fibroblasts that expand beyond the transition zone of the prostate. A general morphological progression pattern of stromal thickening is followed by glandular growth due to secondary branching of existing glands (rather than new ducts budding of the urethra). A better 3-D understanding of budding and branching in human BPH is needed. Questions—such as the susceptibility of different branches to hyperplasia—could be addressed by spatial imaging modalities. A better understanding of mitogenic and morphogenic pathways activated during BPH also is necessary. The group agreed that multiple data types (e.g., genetics, epigenetics, transcriptomics, proteomics, functional immunology) and multiple model systems (e.g., 2-D and 3-D cell lines, fetal prostate explants, tissue regeneration assays, transgenic animals) will be required to develop a spatial and cell-type specific understanding of stromal–epithelial and immune–prostate interactions at each stage of prostate development and disease.

Most likely "first hit." The group agreed the BPH is a multifactorial disease, with several likely first hits: fetal imprinting, stress-induced activation of developmental pathways in interstitial fibroblasts, decreased local androgen production or increased conversion to estrogen or glucocorticoids, systemic or local autoimmunity or inflammation, age-related senescence, glandular leakiness, voiding pressure, urine reflux, genetics, stromal and epithelial stem cell activation, metabolic disease, parasites, microbiome effects, loss of immunoregulation, and some combination of the above.

Anatomical location of BPH. The group discussed reasons that BPH is restricted to the transition zone. This could be due to autocrine activation of proximal stem cells, embryonic reawakening of peri-urethral interstitial fibroblasts by paracrine signaling, or urinary reflux into proximal ducts.

Breakout Session 2

Improved subtyping of patients with BPH/LUTS. Dr. Brooks presented the group's detailed deliberations on patient subtyping. Better stratification of patients will provide biological insights into BPH and aid in identifying clinically relevant pathways. Subtypes should correlate with responses to existing therapies,

presence of symptoms, and alterations to driver pathways that are potential therapy targets. The group agreed that such noninvasive methods as blood and urine collection would be best suited for translating subtypes into clinically actionable information. Patient features identified using the LURN index and other features collected during clinical trials (e.g., psychosocial elements, social determinants of health) should be used for subtyping. Germline SNVs might correlate with risk for symptoms, progression of symptoms, and symptom types. Polygenic risk scores should be developed and used to identify at-risk patients for preventive intervention trials. The group discussed the role of spectrum bias in the collection of patient samples; high-quality tissues are available only from large prostates removed via open prostatectomy. The group reviewed the challenges and opportunities associated with collecting prostate samples during cancer surgeries and the need to screen molecular profiling methods for utility in disease stratification. Bladder subtyping also was discussed. Discernable subtypes of bladder responses to obstruction should be identified and correlated with symptoms, if possible. Bladder–prostate crosstalk is a possible target for therapeutics.

Strategies for tissue acquisition. The group discussed the importance of harvesting bladder, prostate, and other tissue samples from the same patient. The group members noted that having a single donor would be beneficial for deep clinical phenotyping. The group discussed sources of tissue samples:

- Healthy tissues can be harvested from warm autopsies, but these tissues are not accompanied by voiding phenotype information.
- Volunteers can be paid for prostate and bladder biopsies after deep phenotyping.
- Tissues can be harvested during transvesical enucleation procedures, although these patients represent a discrete BPH subtype and often are medicated heavily.
- Bladder samples can be taken from patients undergoing TURPs.
- Samples can be harvested from non-tumor tissue in patients with bladder cancer.
- Specimens can be obtained during biopsies to investigate elevated PSA levels because most of these patients do not have cancer.

The group reviewed challenges associated with sample collection. Standard protocols should be developed for the collection, storage, and disaggregation of high-quality samples. Issues—such as sample contamination with tumor cells, sampling bias associated with needle biopsies, and sample sources for exploratory studies—were discussed. Dr. Brooks emphasized the importance of sample storage infrastructure for these efforts.

Promising biological measures. A detailed anatomical and functional understanding of BPH still is required. Metrics beyond prostate size—such as intravesical projection and transition zone volume and diameter—should be collected. Functional imaging should be used to investigate characteristics, such as differences in bladder neck funneling and urethral elasticity and diameter. Dr. Brooks called for a mechanical engineering approach to modeling the LUT. The group discussed collecting short-term longitudinal data using implanted nanotechnology devices and noninvasive imaging methods for short-term monitoring. Long-term longitudinal data for patients who develop BPH and LUTS can include serial prostate biopsies, serial clinical phenotyping, and serial collection of biofluids.

Tools and methods for clinically useful insights. Dr. Brooks noted that most men have improvement of LUTS after relief of obstruction. The LUT can be assessed before and after treatment to compare responders and nonresponders. Insights from modeling the LUT can be used to inform the anatomical, clinical, imaging, and urodynamic features needed for subtyping. The full spectrum of patients (e.g., large

prostates with symptoms, large prostates with no symptoms, small prostates with no symptoms, small prostates with symptoms) must be profiled.

Breakout Session 3

Novel biomarkers of aging. Dr. Ricke summarized the group's discussion of aging biomarkers, including senescence, fibrosis, inflammation, and cellular mosaicism. He noted that several approaches (e.g., frailty indices, epigenetics, animal models of inflammatory exposures across time, gain of function and loss of function models of senescence) can be used to investigate biological versus chronological age. He emphasized the need for rigorous controls to determine whether biological events associated with BPH exist in "normal" aged animals.

Role of biorepositories and tissue access. The group agreed that more access to tissues and additional biorepositories are needed. Researchers require access to human specimens and animal models to develop grants and perform studies. Models may be available via the Mouse Models of Human Disease group, toxicology databases, and other studies that are not utilizing LUT tissues. Dr. Ricke pointed out that surgeries that are destructive to BPH tissues are becoming more prevalent; obtaining fresh tissue samples might become an issue. He wondered whether immediate efforts should be made to collect and archive high-quality samples while still possible or to obtain samples from other countries.

Latest contributions to modeling basic research. Human physiology and symptoms must be connected to cellular and molecular biology mechanisms. Animal models must represent human aspects of prostate biology and disease (e.g., hyperplasia, smooth muscle tone, fibrosis, drug resistance). The group agreed that naturally aged animals might be the best model for BPH research.

Advantages and limitations of animal models. Comparing anatomy and cell biology across species remains a challenge. This is especially true for comparisons between human and mouse data. Animal models beyond mice (e.g., rats, dogs, non-human primates) are needed but are associated with ethical and cost challenges.

New techniques to understand cellular heterogeneity in BPH. Increased quality control is necessary for single cell technologies, such as scRNA-seq and single cell proteomics. More bioinformatics datasets are needed, especially those that include patient data. Existing methods are sufficient for molecular imaging of BPH and can be combined with preclinical animal models of LUTD.

Breakout Session 4

Dr. Roehrborn summarized the group's directions for approaches to baseline measures:

Prostate measures
- Composition of the prostate
- Multiparametric MRI
- PSA density
- Prostate biopsies

Morphometry
- Assessment of size and shape by ultrasound and/or cross-sectional imaging

Derived measures
- Height, length, and width
- Calculated prostate size and weight assuming specific gravity of 1.06
- Transition zone versus peripheral zone

- Characterization of shape

Urodynamics
- Free flow rate
- Abdominal ultrasound residual urine measurement
- Voiding efficiency and functional bladder capacity
- Home uroflowmetry
- UroCuff
- Formal urodynamic studies (UDS)

Questionnaires
- AUA SI or IPSS
- LURN SI-10 or LURN SI-29
- Sexual Health Inventory For Men
- Male Sexual Health Questionnaire for assessing ejaculatory dysfunction
- Sleep questionnaires
- Frailty questionnaires
- Dementia questionnaire (Patient Health Questionnaire-9)

Endoscopy
- Cystoscopy with bladder wall assessment (trabeculation and diverticula grading)

Bladder wall thickness
- Point-of-care ultrasound (commonly known as POCUS)
- Abdominal ultrasounds
- MRI

Dr. Roehrborn presented several preliminary clinical study and trial designs:

Cross-sectional population-based study
- Flex cystoscopy of men ages 30 to 90
- Graded assessment of trabeculation and diverticula
- Correlation with Q_{max}, transrectal ultrasonography sizing (TRUS), OAB assessment, symptom scores
- Long-term follow-up of patients
- Outcomes: changes to IPSS/LURN SI-10 and SI-29, OAB assessment over time

Natural history study
- Phenotype men with traditional and newer measures
- Follow up in regular intervals with repeat measures
- Determine which measures (alone or in combination) predict deterioration, stable disease, or progression to surgery
- Outcomes: conversion to active treatment based on threshold

Prospective cohort study
- Null hypothesis: intravesical protrusion does not affect outcome of best medical treatment
- Inclusion: typical patients with LUTS/BPH based on IPSS, Q_{max}, TRUS
- Intervention: best medical therapy
- Enrollment stratification by intravesical lobe protrusion size
- Outcomes: IPSS, Q_{max}
- Additional assessment: LURN SI-10 or LURN SI-29 for therapeutic study validation

Sequential Multiple Assignment Randomized Trial design

- Initiate in primary care physician (PCP) office
- IPSS > 8, undergo TRUS or imaging
    - Intravesicular protrusion absent, go on medical therapy, stay with PCP, follow up, and monitor treatment response
    - Intravesicular protrusion present, refer to urologist for management, additional functional tests (UDS, UroCuff)
        - If obstructed, minimally invasive surgical therapies or surgery
        - If unobstructed, back to PCP

CLOSING REMARKS
Tracy Rankin, Ph.D., NIDDK, NIH
Robert Star, M.D., NIDDK, NIH

Dr. Rankin thanked participants for their contributions to the workshop. She encouraged participants to submit proposals to NIDDK and provided a list of NIDDK and NIA staff contacts for discussing these grant proposals.

Dr. Star thanked KUH staff, organizing committee, speakers, and participants for their contributions. He highlighted the paradigm shift in BPH/LUTS patient evaluation that has occurred during the last 20 years. Treatment evaluations have shifted and expanded from metrics focusing on prostate size and symptom scores to deep clinical phenotyping, patient subgroups, other organ systems, assessments of inflammation and fibrosis, and high-throughput molecular and cellular analysis techniques. Dr. Star reviewed cross-cutting themes and basic science and clinical questions that emerged in the workshop, including the definitions of BPH, the ability to connect symptoms to mechanisms, early interventions, and the role of developmental biology. He closed with a call for patient involvement in clinical and research efforts.

ADJOURNMENT
Tracy Rankin, Ph.D., NIDDK, NIH

Dr. Rankin adjourned the meeting at 4:05 p.m.