Acupuncture for Female Interstitial Cystitis/Painful Bladder Syndrome and Its Effect on the Urinary Microbiome: A Randomized Controlled Trial

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A. BACKGROUND AND CLINICAL SIGNIFICANCE:

Interstitial Cystitis/Painful Bladder Syndrome (IC/PBS), affecting nearly 7.9 million US adult women[1], is a complex chronic pelvic pain (CPP) condition characterized by suprapubic pain related to bladder filling and associated with urinary urgency and frequency in the absence of urinary infection or other obvious pathology[2]. Quality of life with IC/PBS is significantly reduced particularly in those with concomitant pain comorbidities[3]. Proposed etiologies have included bladder glycsosaminoglycan (GAG) layer injury with resultant neurogenic inflammation, autoimmune basis with mast cell infiltration, epithelial dysfunction, infectious ([4]) and peripheral and/or central sensitization. The presentation of symptoms can be quite variable[5,6], suggesting that IC/PBS is a multi-factorial disease with overlapping etiologies[7] driven by complex pelvic neural circuitry[8]. Definitive diagnostic criteria for IC/PBS and research measures that correlate with treatment outcomes are lacking[4]. Additionally, etiologic and contributing factors to symptom manifestation are not well defined.

Recent IC/PBS guidelines[9] advocate first and second line treatment directed at pain management. Over 80% of patients with IC/PBS seek complementary and alternative medical (CAM) treatment[10]. There is mounting evidence to support acupuncture as an alternative therapy in various types of chronic pain[11]. Several mechanisms of action have been proposed for acupuncture treatment effect. Various models have implicated cytokines, hormones (eg, cortisol and oxytocin), biomechanical effects, electromagnetic effects, the immune system, and the autonomic and somatic nervous systems (Andew C Ahn, Acupuncture. UpToDate). Current understanding of acupuncture's analgesic effect thought to be associated with neurotransmitters release at both the spinal and supraspinal levels. Recent preliminary evidence documents the efficacy of acupuncture in lower urinary tract symptom reduction in men with chronic prostatitis/CPPS[12]. Yet there is a ***significant knowledge gap*** regarding its effect in women with IC/PBS.

The Urinary Microbiota. Within internal surfaces, which are defined as existing outside the body (e.g. the intestinal epithelium or the vaginal epithelium), there exist commensal microbial communities. These microbiota are believed to be beneficial to human health, facilitating efficient removal of improperly functioning immune cells and protecting the host from pathogen infection[13-15]. The human bladder is a core component of the human urinary tract. It is a hollow muscular organ lined with transitional epithelium, which functions as the storage site for metabolic wastes in the form of urine. Given that the bladder's luminal space is also considered outside the body, it would seem reasonable that a urinary microbiota would be present. Yet, the historic dogma has maintained that urine is "sterile," based on culture-dependent methods of bacterial detection. This paradigm is shifting, however, based on our newly published data noting the presence of a urinary microbiota in adult women without clinical urinary tract infections[16,17]. Furthermore, we have recently discovered that the microbiota of women with overactive bladder (specifically with associated urgency incontinence) is distinguishable from the microbiota of women with stress urinary incontinence. These findings suggest that IC/PBS may be influenced by an alteration to the urinary microbiota. Indeed, recent evidence reports a difference in the urinary microbiome dominated by Lactobacillus in subjects with IC/PBS compared with healthy controls[18]. This work is limited by lack of correlative clinical symptomatology, small sample size and urinary samples that may have been contaminated.

Cytokines and Pain. Upon exposure to a pathogen, dramatic metabolic changes occur within the infected cell. One consequence of these alterations is the initiation of the innate immune response. This immune response is the first line of defense against the infection. It is comprised of cells and mechanisms that defend the host from infection by organisms in a non-specific manner. The innate immune response is initiated immediately upon infection, by the binding of proteins, e.g. toll like receptors (TLR), nucleotide-binding oligomerization domain-containing proteins (NOD), and retinoic acid-inducible (Rig)-like proteins to specific pathogen associated molecules. These proteins can be found on the cell surface, on the surface of internal vesicle and organelle membranes, or freely within the cytoplasm of the cell including cells of the nervous system. Each protein recognizes a different pattern molecule associated with specific types of pathogens. For example, the proteins TLR 2, 4 and 5 recognize bacterial cell surface components lipoteichoic acid (LTA), lipopolysaccharide (LPS) and bacterial flagellin, respectively (24). Binding of bacterial ligands to host receptors activates signaling

pathways that result in the production of pro-inflammatory proteins, e.g. type I interferons, tumor necrosis-factor alpha (TNF-α), interleukin-1 (IL-1) and numerous chemokines.

The inability of the ligand to bind its cognate receptor, the lack of or aberrant activation of the downstream signaling cascades, or decreased production of pro-inflammatory proteins are known to influence neuronal function and pain symptoms (13-14). The central nervous system regulates systemic inflammatory responses to many pathogens including Gram-negative bacteria (15). Afferent fibers from the central nervous system innervate the bladder and the surrounding detrusor muscle. LPS and various cytokines can activate these afferent fibers, consequently stimulating the hypothalamic-pituitary-adrenal anti-inflammatory response (16). The efferent fibers of the central nervous system, which also innervate the bladder and detrusor muscle, then mediate the parasympathetic anti-inflammatory response. It is during this response that the brain modulates the systemic inflammatory pathway (17). Stimulation of these nerve fibers in the presence LPS inhibits the production of tumor necrosis factor (TNF-), potent symmetric inflammatory cytokine. This inhibition is thought to occur via both the nicotinic and muscarinic acetylcholine receptors (17). Conjugation of TLRs and other pathogen-specific sensing molecules stimulate the production of type I interferons, IL-6, IL-10, MCP1, IL-1α and other pro-inflammatory cytokines. Receptors for these molecules are present on the efferent central nervous system nerve fibers.

Results of studies of acupuncture have suggested that analgesia is mediated by regulating production of cytokines including pro and anti-inflammatory mediators[19,20]. Various models have implicated cytokines, hormones (eg, cortisol and oxytocin), biomechanical effects, electromagnetic effects, the immune system, and the autonomic and somatic nervous systems (Andew C Ahn, Acupuncture. UpToDate, updated 12/2013).

Prior research has also noted a differential expression of several urinary cytokines between women with interstitial cystitis and asymptomatic controls, pointing to the relationship between their expression and symptom development[21,22]. Irritative bladder symptoms in IC/PBS may result by aberrant signaling from the central nervous system due to the presence cytokines and other pathogen-associated molecules during initiation of the innate immune response.

The reports that acupuncture's influence may be partially expressed through immune mechanisms are reinforced by the understanding that beta endorphin and met-enkephalin seem to be mediators between the central nervous system and immune system. The two substances have been shown to enhance natural killer cell activity and met-enkephalin increases in vitro T-lymphocyte rosette formation from human T-lymphocytes (Josef M. Helms. Acupuncture energetics **1995** | ISBN-10: **1572507063** | ISBN-13: **978-1572507067** | Edition: **1st)**.

It seems logical to theorize that acupuncture treatment may lead to urinary microbiome change.

We propose that changes in bladder microbiota leading to this enhanced cytokine response may result in symptoms associated with IC/PBS and that acupuncture, by modulating cytokine response, in turn has effect on the urinary microbiome.

Our ***long term goal*** is to determine pelvic pain mechanisms that will inform clinically-relevant classification and evidence-based treatment of women with IC/PBS and CPP. ***The short term goal*** of this application is to determine the safety, tolerability and efficacy of acupuncture in women with IC/PBS as a neuromodulative treatment and to characterize the urinary microbiome before and after acupuncture treatment. Our approach will advance the understanding of the contribution and consequences of peripheral pelvic nociception in IC/PBS. Our ***central hypothesis*** is that women with IC/PBS will benefit from acupuncture compared with sham treatment and acupuncture will have an effect on the urinary microbiome.

This proposed work **impacts** public health with contributions of rigorous clinical and translational methods, suitable for future NIH clinical research networks, to evaluate and assess treatment outcomes in women with IC/PBS. It is likely that these methods will have impact beyond this group of affected patients, such as CPP related to irritable bowel syndrome, vulvodynia, pregnancy, and endometriosis.

B. INNOVATION: We believe that the proposed research is innovative, as current pharmacologic strategies for IC/PBS have limited success and/or problematic side effects, yet acupuncture has not been studied in this population to date despite its proven efficacy in other chronic pain disorders[11]. In addition, no study to date has correlated the urinary microbiome in IC/PBS with clinical characteristics or with response to acupuncture treatment.

C. STUDY POPULATION: Women presenting with IC/PBS at the Urogynecology/Urology clinics at Loyola University Medical Center will be approached for research participation. A certified research team member will explain the purpose and procedures of the study. Potential participants who meet inclusion criteria and agree to participate will indicate their consent by signing the informed consent document. Consistent with prevalent research regulations and policies, a signed copy of the research consent document will be given to the participant and the investigator will keep the original research consent document. Enrolled participants with IC/PBS must meet all the following eligibility criteria: **Inclusion**: 1) Females, age 21 to 65 years (no racial/ethnic restrictions); 2) Symptoms of urinary frequency, urgency and suprapubic/bladder pain for > 6 months; 3) Generally stable health 4) An average bladder pain score of at least >3/10. **Exclusion**: 1) Patients with pacemaker or other neurostimulator (gastric/spinal) 2) History or current symptomatic urethral stricture, cystitis caused by tuberculosis, radiation therapy or Cytoxan/cyclophosphamide therapy; 3) Prior augmentation cystoplasty or cystectomy; 4) Systemic autoimmune disorder (such as Crohn's Disease, Ulcerative Colitis, Lupus, Rheumatoid Arthritis, or Multiple Sclerosis); 5) Systemic neuromuscular disease known to affect the lower urinary tract; 6) History of urogenital cancer (with the exception of minor skin cancer); 7) Current or imminent planned pregnancy/recent delivery <6 months; 9) Current pelvic floor physical therapy; 10) Current use of opioid medications (short or long acting) for pain; 11) Abdominal or pelvic surgery within the last 6 months12) No symptoms or diagnosis of UTI within the past 3 months

Additionally 20 control women will be recruited for primary comparison of urinary microbiome data with IC/PBS cases. Inclusion for controls: : 1) Females, age 21 to 65 years (no racial/ethnic restrictions); 2) No history of or current bladder or pelvic pain; 3) Generally stable health 4) English speaking **Exclusion**: 1) Patients with pacemaker or other neurostimulator (gastric/spinal) 2) History or current symptomatic urethral stricture, cystitis caused by tuberculosis, radiation therapy or Cytoxan/cyclophosphamide therapy; 3) Prior augmentation cystoplasty or cystectomy; 4) Systemic autoimmune disorder (such as Crohn's Disease, Ulcerative Colitis, Lupus, Rheumatoid Arthritis, or Multiple Sclerosis); 5) Systemic neuromuscular disease known to affect the lower urinary tract; 6) History of urogenital cancer (with the exception of minor skin cancer); 7) Current or imminent planned pregnancy/recent delivery <6 months; 9) Current pelvic floor physical therapy; 10) Current use of opioid medications (short or long acting) for pain; 11) Abdominal or pelvic surgery within the last 6 months. 12) No symptoms or diagnosis of UTI within the past 3 months. These women after screening for eligibility will complete a basic demographics form for controls (see attached) and provide one voided clean catch urine specimen. They will not be required to undergo physical examination or vaginal swab or complete any other additional questionnaires. Women will be recruited from the LUC Health Sciences campus and will be provided a parking sticker for their participation.

D. DESIGN OVERVIEW: This prospective randomized single blinded study will compare the effect of true acupuncture compared with sham/minimal acupuncture in women with IC/PBS (n=30). Symptomatic IC/PBS participants will not be asked to stop taking standing medications such as neurological agents in order to participate in the study. All current medications will be recorded and will measured using the Medication Quantification Scale (MQS III)[23,24]. They will be asked to refrain from use of short-acting opioids/acetaminophen/non-steroid anti-inflammatory medications during study visits. IC/PBS participants will engage in 7 acupuncture sessions once weekly. Initial visit would assess participant's response to needle insertion. Standard classical/true acupuncture treatment will be administered during 6 following visits to treatment group (n=15) and sham/minimal acupuncture will be administered to control group. Menstrual cycle time points will be recorded for each visit. Clean catch voided urine samples and vaginal swab will be obtained prior to initiation of intervention, on completion of intervention and 12 weeks post onset of study visits.

E. RESEARCH STRATEGY:

Specific Aim 1: To determine the safety and tolerability of acupuncture in women with IC/PBS.
Hypothesis 1*: Acupuncture treatment (true and sham) will be safe and tolerable in women with IC/PBS.*

Specific Aim 2: To determine if acupuncture is effective in reducing pain in women with IC/PBS compared to sham treatment.
Hypothesis 2*: True acupuncture treatment will be effective in the treatment of pain in women with IC/PBS compared with sham/minimal treatment.*

Specific Aim 3: To determine the association between acupuncture responders and the urinary microbiome.
Hypothesis 2*: Acupuncture responders will exhibit change in the urinary microbiome compared with nonresponders after acupuncture treatment.*

STUDY DESIGN/METHODS:

Acupuncture Component:
This is a randomized, controlled, single-blinded, pilot trial to study the feasibility, safety, and efficacy of acupuncture in IC/PBS women. Women will be recruited from the patient population of the Loyola University Medical Center who meet criteria for diagnosis of IC/PBS. This trial had 2 arms comparing the treatment of IC/PBS symptoms with acupuncture or sham/minimal acupuncture (placebo).Thirty (30) patients with IC/PBS will be enrolled as study participants into the study. Fifteen (15) will be allocated in the treatment classical acupuncture + medical management of IC and fifteen (15) will be allocated in the control sham/minimal acupuncture + standard medical treatments of IC. The acupuncturist will interview each patient and perform an exam. A standardized acupuncture treatment will be assigned, and both groups will receive 7 acupuncture treatments that follow a standardized protocol on classical acupuncture points, with or without mild electrical stimulation versus sham/minimal acupuncture. Acupuncture needles are single use, sterile and disposable. Standard acupuncture treatment protocol will include 4 gates plus GV 20 to reduce anxiety and help with relaxation and to assess acupuncture naïve patient's response to needles during their first acupuncture encounter. Subsequent visits would include administration of curious meridian Chong Mo paired with Yang Ming. 4 Hz low level electrical stimulation will be applied. Control group will receive sham/minimal acupuncture with low level electrical stimulation. The sham intervention (also described as minimal intervention) will use superficial needle insertion at body locations not recognized as true acupoints. Patients will be explained that various acupuncture treatment protocols will be tested including "minimal acupuncture", therefore, the control group will not be aware of receiving sham acupuncture. These described acupuncture treatments are well accepted treatment protocols for women with pelvic pain and bladder complaints.
Acupuncture or sham needle treatments will be performed once a week for 7 weeks. All subjects will be reassessed following the 7-week intervention phase and then 12 weeks from initiation of study. All subjects will be treated by single acupuncture practitioner (Dr. Bresler) who is also a board certified urologist specializing in IC/PPS and is a current associate member of American Academy of Medical Acupuncture and board eligible for the American Board of Medical Acupuncture.

Clinical Measurements:
The following clinical parameters will be assessed in all participants at baseline and repeated at follow-up 12 weeks after initiation of PFPT:
Demographics: age, self-reported race and ethnicity, vaginal parity, body mass index, menopausal and hormone replacement status, prior treatment for IC/PBS and other pelvic floor disorders, tobacco and alcohol use, prior medical and surgical history, medications including pain medications measured by the Medication quantification scale (MQS III)[23,24]), allergy history, social history
Standardized physical exam: Abdominal exam with vaginal assessment of pelvic floor muscle function generalized muscle tenderness exam[25]
Pain location, duration, intensity: Brief Pain Inventory – Short form (BPI-SF)[26], VAS pain scale[27], Short Form McGill Pain Questionnaire[28], Body Pain Diagram
Pain functional impact: Pain disability index[29]; Patient Global Response Assessment (GRA)[30]

Urinary/Pain Symptoms: Female Genitourinary Pain Index (FGuPI)[31], Interstitial Cystitis Symptom Index and Problem Index (ICSI, ICPI)[32]
Sexual Dysfunction: Female Sexual Function Index[33]
Psychological impact/Coping style: Pain Catastrophizing Scale (PCS)[34], Beck Depression Inventory, second version (BDI-II)[35], Beck Anxiety Inventory (BAI)[36]
Clean catch voided urine specimen: for urinalysis, culture and microbiome analysis
Vaginal Swab: to exclude vaginal bacteria from voided urine specimen.

Urinary Microbiome Approach:
In order to elucidate changes in the bladder's microbiota and to understand how the host's immune response may contribute to IC/PBS, we propose a multidisciplinary approach that blends molecular biology, immunology, genomics and clinical assessment. We will use *de novo* sequencing to determine the composition of the bacterial community present in the urine of women with IC/PBS undergoing acupuncture treatment. Urine will be collected at three time points via clean catch voided urine specimen and vaginal swab: at baseline, post intervention completion and 12 weeks after the onset of the intervention.

Preliminary Data: In April, 2012, we published data concluding that bacteria are present in the bladders of culture-negative women without symptoms of UTI[16]. More recently, we completed an analysis of 13 urine samples from individual women with either stress urinary incontinence (SUI) or urgency/frequency (U/F) or both. From this preliminary analysis, we found that the urine microbiota differs between patients with predominately SUI symptoms compared to those with predominately U/F symptoms. ***Figure 1*** shows the illustrative results of our microbiota analysis of these 13 samples. The composition of the microbiota for each patient was determined by sequencing 150 nucleotides of the DNA encoding for the RNA molecule found within the small unit of the bacterial ribosome. These sequences identify the bacterial species present in each patient's urine. Principle component analysis (PCA) was used to determine the amount of difference between the groups of patients. This graph depicts the result of this examination, which suggests the microbiota of women with SUI or SUI>U/F significantly differ from that of women with U/F or U/F>SUI. This difference has the potential to be used as biomarker for various types of bladder dysfunction. Our results clearly indicate that the urine microbiota may be related to the etiology of different UI subgroups. Subsequently, we hypothesize that measurable biological changes in the urinary microbiota and/or urinary cytokine levels correlate with irritative bladder symptoms in women with IC/PBS.

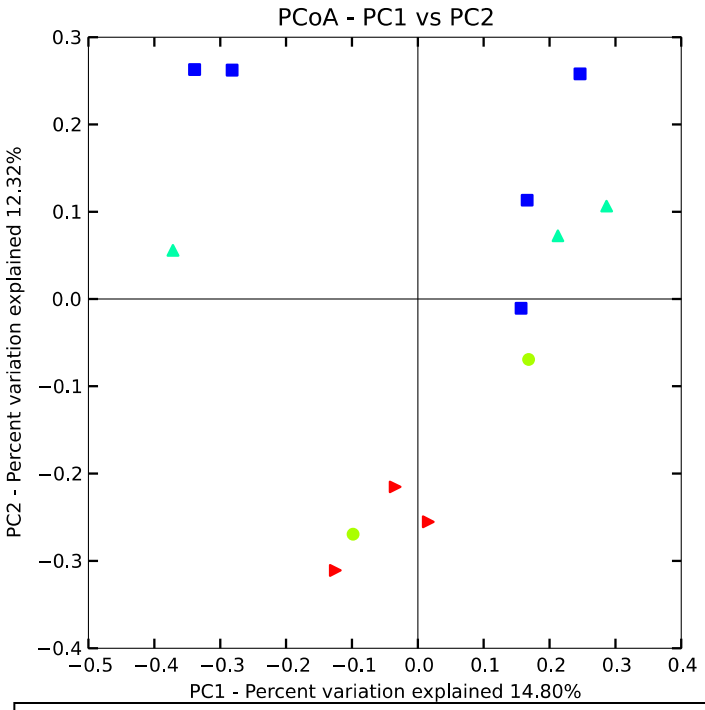

Figure. 1: PCA- patients of different UI subgroups can be distinguished by their microbiota. SUI (orange) and SUI >U/F (lime) are different than U/F (aqua) and U/F >SUI (blue) along Axis 2.

Analysis of Urinary Bacterial Communities by* De Novo *Whole Genome DNA Sequencing (WGS) Rationale. Most microbiome studies define a catalog of bacterial species present in the microbial community on the basis of the sequence of one or more variable regions of the 16S ribosomal RNA (16S rRNA) gene. This assay is based upon a similarity between what is present in the sample and what bacterial species are already known. Therefore, many bacterial species present in the community may not be identified. *De novo* whole genome sequencing eliminates this bias. Genome assembly is based upon the presence of overlapping sequence between the assayed DNA fragments. Thus, the entire bacterial community can be identified, exponentially increasing our understanding of the relationship between the bladder's bacterial community and

OAB. The rapidly decreasing cost of WGS makes this more comprehensive approach feasible and thus desirable.

Sequencing Approach. Bacterial DNA will be isolated from 0.5 ml of each urine sample, randomly fragmented, modified for and sequenced using Illumina's MiSeq technology. This technology allows for sequence of more than 200 nucleotides of DNA from each strand of the DNA duplex to be generated, facilitating the downstream bioinformatics analysis. WGS by MiSeq technology is expected to generate more than 100X coverage, the average number of sequences representing a given nucleotide from the generated sequence, for each sample. This magnitude of data allows for samples to be multiplexed, individually barcoded, and sequenced at once.

***Bioinformatics Approach*.** Quality control & de-multiplexing, the separation of each individual sample from the group based upon their barcode, the sequence data can be readily achieved with MiSeq Reporter software from Life Technologies (current version: 2.0). Only high quality reads (≥Q20) will be kept for analysis. Reads will be aligned and assembled using open source algorithms such as the AMOS and CABOG assembler packages to bioinformatically generate the genome of a specific bacterium.

Table 1. **Schedule of measures for the proposed study.**

Schedule of Measures:	**Baseline**	**Post Acupuncture Completion**	**12 Weeks from Baseline**
Demographics	X	X	X
Questionnaires	X	X	X
Physical exam	X	X	X
Urine Specimen	X	X	X

F. DATA ANALYSIS/SAMPLE SIZE ESTIMATION:
The primary objective is to compare the mean BPI-SF worst pain score at following acupuncture between groups. All other analyses are secondary outcomes. A separate linear mixed effects model will regress each pain score outcome on group (minimal versus electro-acupuncture), time (baseline, end of treatment, or end of follow-up), and a group by time interaction. Random intercepts will be included to account for within-patient correlation. Adjusted means and standard errors will be estimated, and main effects and the group by time interaction will be formally tested within the mixed model. A similar linear mixed effects model will regress relative abundance of uropathogen on group, time, and the group by time interaction. Based on the prior work of Crew KD et al.[37], a sample size of 30 participants (15 per arm) was calculated to yield 90% power to detect a reduction of 2.5 on the BPI-SF worst pain item at a 5% significance level, assuming a standard deviation of difference in score between treatment groups of 2.5 points. This includes a dropout rate of 10% A reduction of at least 2 points on the BPI-SF worst pain item is considered to be a clinically meaningful decrease based on the literature[38]. All statistical analyses will be two-sided and performed using SAS 9.4 (SAS Institute, Cary, NC).

G. FUTURE DIRECTIONS: The impact and potential benefits of identifying new nonpharmacologic treatment in women with IC/PBS will be critical to reduce disease severity and improve quality of life across female IC/PBS and all CPP subtypes. These same methods will be employed for future clinical trials in IC/PBS and other CPP subtypes. In future studies, participants could be reassessed monthly for 6 months after post-intervention assessment (6 months after completing the treatment protocol), and subjects who relapse could receive one to two booster/maintenance acupuncture sessions with additional studies performed on the urinary microbiome.

H. TIMELINE:

Research Activity	Year 1 (2013 – 2014)			
	Qt 1	Qt 2	Qt 3	Qt 4
Recruitment and Evaluation of Participants	X	X	X	
Data Collection	X	X	X	
Data Analysis/Manuscript writing/editing			X	X

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