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Urinary Biomarkers in Women with Refractory Urgency Urinary Incontinence Randomized to Sacral Neuromodulation versus OnabotulinumtoxinA Compared to Controls

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Abstract

Purpose—To measure urinary biomarker levels in women with refractory urgency urinary incontinence (UUI) and controls at baseline and 6 months post treatment from sacral neuromodulation (SNM) or intradetrusor injection onabotulinumtoxinA and to assess association of baseline biomarkers to post treatment UUI episodes (UUIE) and overactive bladder (OAB) symptom bother outcomes.

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Materials and Methods—First morning urines were collected from consented trial participants and age-matched women without UII. Biomarkers reflecting general inflammation, neuro-inflammation, afferent neurotransmitters, and tissue remodeling were measured using standardized ELISA and activity assays as appropriate. Symptom bother was assessed by Overactive Bladder (OAB) Questionnaire and UII episodes by bladder diary. Linear models were used to examine differences in mean biomarker levels, and change in UIIE and symptom bother between baseline and six months. A p-value 0.01 represented modest evidence of a potential association and 0.004 moderate evidence of an association with outcomes.

Results—Baseline biomarker levels differed little between cases and controls, except tropoelastin ($p=0.001$) and N-terminal telopeptide collagen type 1 (NTx, $p<0.001$). Changes in biomarker levels 6 months post intervention included decreases in collagenase ($p<0.001$) in both treatment groups and increases in interleukin-8 ($p=0.002$) and matrix metalloproteinase-9 ($p<0.001$) in the onabotulinumtoxinA group. Higher baseline CGRP across both treatments ($p=0.007$) and nerve growth factor in the onabotulinumtoxinA arm ($p=0.007$) were associated with less reduction in OAB symptom bother.

Conclusions—Refractory UII is a complex condition. These data suggest that matrix remodeling and neuropeptide mediation may be involved in its pathophysiologic mechanisms and response to treatment.

Keywords

refractory urgency urinary incontinence; urinary biomarker; sacral neuromodulation; onabotulinumtoxinA

Introduction

Urgency urinary incontinence (UII) is a symptom characterized by sudden unpredictable urine loss with a sense of urgency affecting women world-wide with increasing prevalence with age and significant impact on quality of life.¹ Refractory UII includes women who fail multiple treatments including medications and behavioral therapy. Treatment for these women can include intravesical botulinum toxin injections (onabotulinumtoxinA) and sacral neuromodulation (SNM).

Targeted effective treatment options are hampered by the absence of reliable biomarkers that could aid diagnosis and predict treatment outcomes. While urinary nerve growth factor (NGF) has been reported increased in patients with overactive bladder (OAB) compared to controls^{2–4} and onabotulinumtoxinA injections resulted in decreased urinary NGF post treatment⁴, these studies are often limited by large variability among subjects, lack of age-matched controls, and differences in methodology of urine collection, processing, and normalization. Histological analyses of bladders impacted by detrusor overactivity demonstrate that both treatments may reverse detrusor muscle hypertrophy and bladder fibrosis presumably due to alterations in the synthesis and degradation of bladder wall matrix components.^{5–8}

Given the high biologic plausibility that neuro-inflammation, increased activation of afferent neural pathways, and tissue remodeling have roles in UII pathogenesis and treatment

response, we hypothesized in this exploratory and hypothesis generating study that NGF levels would be higher in women with refractory UUIE compared to asymptomatic controls based on the literature at study design. We also hypothesized that urinary markers of general inflammation, neuro-inflammation, afferent neurotransmitters, or tissue remodeling may differ in women with refractory UUI relative to age-matched controls. Further, we assessed whether baseline urinary biomarker levels may be associated with clinical response to onabotulinumtoxinA or SNM treatment.

Material & Methods

Subjects

The ROSETTA Urinary Marker (RUM) study was a planned exploratory supplementary study to the Refractory Overactive Bladder: Sacral Neuromodulation versus Botulinum Toxin Assessment (ROSETTA, ClinicalTrials.gov number, NCT01502956) trial, a 9-center open-label randomized trial of SNM or 200 units onabotulinumtoxinA in women with idiopathic refractory UUI. Details of the ROSETTA trial design and methods have been previously published⁹ and the results recently published.¹⁰ The institutional review board of each clinical site and coordinating center approved the protocol. All participants provided written informed consent. The RUM protocol was written with *a priori* consideration of the REporting recommendations for tumour MARKer prognostic studies, (REMARK) guidelines specifically with respect to collection and processing of urine samples, biomarker assays and analysis plan.¹¹

Participants in the RUM study who were “cases” were ROSETTA subjects recruited from June, 2012 until May, 2014 with refractory UUI defined as persistent UUI symptoms despite at least one supervised behavioral/physical therapy and a minimum of two anticholinergic medications. Major inclusion criteria included a minimum of six UUI episodes (UUIE) on a baseline 3-day bladder diary, absence of UUI medications 3 weeks prior to baseline evaluation, and urodynamic assessment within 18 months prior to randomization. Women with relevant neurologic diseases, history of using either of the two study interventions or elevated post-void residuals were excluded. Cases were stratified by age and randomly assigned 1:1 in permuted blocks to SNM (InterStim®, Medtronic, Minneapolis, MN) or 200 U of onabotulinumtoxinA (Botox A®, Allergan, Parsippany, NJ). Daily UUIE were recorded on monthly 3-day bladder diaries over 6 months. OAB symptom bother was assessed at baseline and monthly for 6 months using the OAB Questionnaire Short Form (OABq-SF).⁹

Control subjects were also recruited for participation in this study. Specifically, controls were non-pregnant women 21 years with no complaints of UUI as determined by an answer of “No” to the question, “Do you usually experience urine leakage associated with a feeling of urgency, that is, a strong sensation of needing to go to the bathroom?” over the previous 3 months using adequate contraception, and no current or prior use of OAB medications. Major exclusion criteria included: (1) systemic neurologic conditions or bladder conditions that would affect function, (2) stress incontinence or prolapse surgery within 6 months, (3) Stage 3 or higher pelvic organ prolapse (POP), or protrusion through the vaginal opening per patient report, (4) hematuria, (5) untreated urinary tract infection

(UTI) or (6) prior use of either study therapy for treatment of UUI. To limit potential for confounding variables, controls were recruited into age-specific strata using an estimated frequency matching strategy.

Standardized demographic, clinical data and key procedural elements were collected.

Baseline dates for the controls (date of first urine collection) forecasted target study dates at 3 months $\pm$ 2 weeks and 6 months $\pm$ 4 weeks.

Urine Processing

Subjects were instructed to void first-morning clean-catch urine samples in 120 milliliter (mL) specimen cups and place on ice (0° C) or in the refrigerator (2–4° C) prior to taking to the clinic on a cold pack that same morning. Every effort was taken to limit the collection-to-processing time interval. Specimens absent for hematuria and leukocytes by dipstick were kept on ice or at 4° C for short times undisturbed until processed (within 2–4 hours). After decanting, the urine was centrifuged (1000 g $\times$ 10 minutes) at the clinical site, and shipped on an ice pack for overnight delivery and receipt within one day of collection to a central laboratory (University of Texas Southwestern Medical Center, Dallas, TX) where specimens were aliquoted and stored at –80° C until analysis.

Biomarker Assays

Potential urinary biomarkers of interest included indicators of general inflammation, neuro-inflammation, afferent neurotransmitters, and tissue remodeling (Table 1). Biomarker results (average of duplicates) were normalized to mean urinary creatinine (milligram,mg/deciliter,dL). To avoid potential problems with batching of samples, (i) all samples were analyzed after collecting the final 6 month time point with all 3 time points on the same plate, (ii) controls and cases were analyzed on the same plate (not batched), and (iii) each biomarker was analyzed within the same time frame. The number of samples requiring repeat analysis was <4.5%.

Sample Size

This study was powered to test differences in NGF normalized to urinary creatinine (NGF pg/Cr ng) with a Type I error rate of 0.05, with p-values for all other tests reported descriptively. A planned sample size of 265 ROSETTA participants was used, which provided 90% (80%) power to demonstrate a 50% (44%) reduction, respectively between baseline and follow-up NGF pg/Cr mg associated with treatment. The planned 50 controls combined with the projected 265 participants provided power of greater than 0.99 to demonstrate a difference in NGF/Cr levels between controls and cases consistent with differences previously described.^{3,12}

Statistical Analysis

Demographic characteristics were summarized separately for cases and controls; differences between groups were assessed using chi-squared and t-tests. All biomarkers except collagenase were log transformed prior to analyses. General linear models compared mean levels of biomarker measures between cases and controls at baseline, both unadjusted and

adjusted for body mass index (BMI), functional composite index, menopausal status, and history of recurrent UTI.

Linear mixed models examined differences in mean biomarker levels before and after treatment among cases and compared differences in the two treatment arms across time with all analyses adjusted for site and age stratum. Linear models with either change in OAB symptom bother score or change in UIIE between baseline and 6 months as the outcome and biomarker metrics as predictors. Only biomarkers between baseline and six months found to be associated with outcomes with $p \leq 0.10$ in univariate analyses were included (Interleukin [IL]-8 and CGRP for change in UIIE; NGF, IL-8, CGRP, Substance P, and matrix metalloproteinase[MMP]-9 for change in OAB symptom bother).

Analyses addressed five broad biomarker-related research questions (case and control differences, changes after treatment with SNM or onabotulinumtoxinA, association with UIIE changes, and association with symptom bother changes). Because analyses were considered hypothesis generating, no adjustments were made for multiple comparisons, and p-values should be interpreted accordingly. To balance between under- and over-reporting biomarker associations, this manuscript describes biomarker associations with a p-value between 0.004 and 0.01 as having modest evidence of a potential effect for the specific question and a p-value of 0.004 or less (which represents a Bonferroni correction at the question level) as having moderate evidence. Analyses were performed with the use of SAS software, version 9.4 (SAS Institute, Cary, NC).

Results

Demographics of cases and controls

Asymptomatic women (N=54) had lower BMI (27.2 ± 5.7 vs 32.2 ± 8.2 , $p < 0.001$) and were less likely to categorize themselves as menopausal (72% vs 85%, $p = 0.006$, Table 2).

Women with refractory UUI (N=260) had a higher functional comorbidity index (3.8 ± 2.3 vs 1.9 ± 1.9 , $p < 0.001$) and were more likely to report a history of recurrent UTIs (13% vs 2%, $p = 0.02$).

Baseline cases vs controls

Of 16 candidate biomarkers, 11 were measureable in 80% of the total urine samples and were analyzed. Biomarkers at baseline, 3 and 6 months following treatment are shown in Table 3. No baseline differences were found between cases and controls in NGF and BDNF, IL-6 and IL-8, CGRP and Substance P or MMP-2, MMP-9 and collagenase levels.

Interestingly, baseline urinary tropoelastin and cross-linked N-telopeptides of type I collagen (NTx) levels showed moderate increases in cases compared to controls (both $p \leq 0.001$).

Controls over time

To gain insight into variability of biomarker levels among control subjects as a function of time, biomarkers were assessed baseline and at 3 and 6 months. Biomarker levels were stable over the observation period of 6 months.

Cases post intervention at 6 months

Overall, modest evidence of an increase in IL-8 level ($p=0.01$) was observed 6 months post intervention. Evidence of increase in IL-8 (modest, $p=0.002$) and MMP-9 (moderate, $p<0.001$) levels were noted following onabotulinumtoxinA treatment. In contrast, urinary collagenase activity decreased proportionately in both treatment groups 6 months after intervention ($p<0.001$) without differences between treatment groups ($p=0.75$).

Association of Baseline Urinary Biomarkers to Clinical Outcomes

Models (Table 4) revealed greater baseline UUIE ($p<0.001$), but no other biomarkers to be associated with greater reduction of UUIE at 6 months. Higher baseline CGRP ($p=0.007$) levels were associated with less OAB symptom bother improvement at 6 months with both treatments. Higher baseline NGF levels in the onabotulinumtoxinA arm and higher baseline MMP-9 in the SNM arm were associated with less improvement in OAB symptom bother at 6 months ($p=0.007$, $p=0.004$, respectively).

Discussion

Similar to previous reports, women with refractory UUI were more likely to have elevated BMI and increased functional co-morbidities compared to age-matched controls.^{13,14} In contrast to previous studies,^{2,3,4} after controlling for these baseline differences, baseline levels of urinary neurotrophic factors, NGF and BDNF, were similar in controls and women with refractory UUI. Multiple pro-inflammatory cytokine levels and afferent neurotransmitter levels in urine were also similar between the two groups. However, matrix remodeling markers, tropoelastin and NTx were moderately higher in cases than controls.

Deposition of collagen and elastin occurs commonly in pathologic processes of the bladder, leading to fibrosis, loss of bladder compliance and reduced bladder capacity.^{15–16} These tissue reactions are often accompanied by UUI symptoms.¹⁶ Increased urinary levels of soluble tropoelastin, a precursor of elastin, may indicate ongoing tissue remodeling.¹⁷ While urine levels of NTx are commonly used to reflect progression of bone resorption, especially in postmenopausal women¹⁸ they can also be a generalized marker of connective tissue turnover. Increased levels of NTx in age-matched women with UUI compared to those without UUI is likely to reflect processes other than bone remodeling since the UUI patients were heavier than controls. Together, increased baseline tropoelastin and NTx in women with refractory UUI may reflect ongoing connective tissue remodeling in the lower urinary tract, but other tissue degradation processes cannot be excluded.

The profile of urinary biomarkers six months post-intervention reflected modest or moderate differences compared to baseline values. Although stable in urine from controls, modest increase in IL-8 level and moderate increase in MMP-9 were detected in cases treated with either onabotulinumtoxinA or SNM. IL-8, initially described as a neutrophil and lymphocyte chemoattractant¹⁹, stimulating migration toward sites of infection/inflammation, has subsequently also been identified as a proangiogenic agent and a modulator of collagenase secretion.²⁰

The relative increase in MMP-9 from baseline to 6 months after treatment with onabotulinumtoxinA, but not SNM, is intriguing. In contrast with most other collagenases, MMP-9 is also a major elastase²¹ that may be involved in reversing detrusor muscle hypertrophy and bladder fibrosis via collagen/elastin degradation.^{7,22} Persistent long-term increases in urinary IL-8 and MMP-9, together with simultaneous decreases in collagenase activity, suggest that in the case of the onabotulinumtoxinA treatment group, we may be measuring remodeling of the bladder wall. Increased baseline levels of urinary tropoelastin combined with an absence of urinary MMP-1 or MMP-13 may indicate that remodeling of the elastic fiber network of the bladder wall is the primary extra-cellular matrix target of onabotulinumtoxinA.

Higher CGRP at baseline was associated with less reduction in symptom bother in both treatment groups. CGRP is a potent neuropeptide involved in the transmission of pain and bladder irritation. In women with detrusor overactivity, onabotulinumtoxinA may inhibit detrusor muscle contractions through blockage of CGRP²³, glutamate and substance P from sensory neurons.²⁴ Increased release of CGRP from afferent terminals at baseline may inhibit the ability of SNM and onabotulinumtoxinA to reduce symptoms of UUI.

Multivariable analysis showed that patients with relatively increased NGF levels at baseline did not respond as well to onabotulinumtoxinA in terms of reduction in OAB symptom bother. The functional remodeling of afferent nerves may be driven by a variety of neurotrophic factors including NGF. Increased expression of urinary NGF has been shown in patients with OAB, detrusor overactivity and UUI compared to controls.^{2,3} Additional clinical studies determined that onabotulinumtoxinA injections decreased bladder urinary NGF post treatment.^{4,12} There may be a number of reasons we did not find increased levels of NGF at baseline in women with refractory UUI compared to other studies. Several recent reviews regarding NGF and other biomarkers have noted that prior studies have reflected lack of: control groups, age-matched controls, standardized specimen collection as well as a lack of prospective data.^{25,26,27} They describe variations in assay approach including lack of normalization to urinary creatinine and the use of an assay kit which was demonstrated to have IgG cross-reactivity (see Promega, Madison, WI, USA, Table 1). There was also little explanation regarding how biomarkers below the lower level of detection were managed. All of these issues are addressed in our study. Our control levels more closely aligned with those noted in Ghoniem et al²⁸ and Pennycuff et al²⁹, who also noted no differences in NGF in OAB cases and asymptomatic controls further emphasizing the importance of controls in these studies.

Strengths of this study include that it was a hypothesis driven and hypothesis generating, prospective, large scale, longitudinal study involving a single-laboratory with standardized evaluation of urinary biomarkers in women with refractory UUI before and after two standard treatment modalities. First-void morning urines were used for analyses, gold standard for biomarker measures providing the least variability in protein concentration.¹¹ A comprehensive analysis of major biomarkers reflective of changes in inflammatory, neuro-inflammatory, afferent, and fibrotic pathways pre- and post-treatment was performed and analysis was performed only on those biomarkers where 80% or more of samples had a detectable level within the standard curve.

Limitations include having insufficient power to fully adjust for the multiple comparisons across the full range of analyses conducted. While p-values are presented without adjustment, we did use a Bonferroni-type correction at the research question level in our interpretation of the data to provide protection against identifying an excess of potential biomarker relationships. Still definitive conclusions among these associations should be viewed cautiously.

Conclusions

These data suggest that matrix remodeling, a novel new finding, and neuropeptide mediation are potential mechanisms involved in the pathophysiology of refractory UUI and response to treatment. NGF levels were not different between women with UUI refractory to medical therapy and asymptomatic controls. Further studies, including comprehensive tissue analyses, will be important to more fully characterize the physiologic mechanism of refractory UUI and targeted therapies.

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Abbreviations

UUI	urgency urinary incontinence
SNM	sacral neuromodulation
NGF	nerve growth factor
OAB	overactive bladder
RUM	ROSETTA (Refractory Overactive Bladder: Sacral Neuromodulation v. BoTulinum Toxin Assessment) urinary marker
REMARK	REporting recommendation for tumor MARKer
UUIE	urgency urinary incontinence episode
OABq-SF	OAB Questionnaire-Short Form
mL	milliliter
dL	deciliter
NGF/Cr	nerve growth factor for creatinine
BMI	body mass index
UTI	urinary tract infection
IL	Interleukin
MMP	matrix metalloproteinase

BDNF	brain-derived neurotrophic factor
NTx	N-terminal telopeptide type 1 collagen
GM-CSF	granulocyte macrophage colony stimulating factor
TNF	tumor necrosis factor
HRQL	health related quality of life
UDI	Urogenital Distress Inventory
IIQ	Incontinence Impact Questionnaire

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Table 1

Urinary Biomarkers and Assays

Assay ^{*†}	Method	Detection Range	Source
Urinary Creatinine	QuantiChrom™ Creatinine Assays	0.1 – 300 mg/dL (1–3000µg/mL)	BioAssay Systems, Hayward, CA, USA
<u>General Tissue Inflammation</u>			
IL-1β [*]	5-plex magnetic polystyrene bead-based multiplex immunoassay	2.8–8,800 pg/ml	R&D Systems, Minneapolis, MN, USA
IL-6		0.7 – 4,950 pg/ml	
GM-CSF [*]		2.8 – 17,700 pg/ml	
TNF-α [*]		1.1 – 6,700 pg/ml	
IL-8		1.7 – 10,500 pg/ml	
<u>Matrix Remodeling</u>			
MMP1 [*]	4-plex magnetic polystyrene bead-based immunoassay	3.9 – 55,450 pg/ml	R&D Systems, Minneapolis, MN, USA
MMP2		12.7 – 16,900 pg/ml	
MMP9		17.8 – 77,600 pg/ml	
MMP13 [*]		76.6 – 335,000 pg/ml	
Tropoelastin	Fastin elastin kit by ELISA	2 – 70 mg/ml	Biocolor Ltd., Northern Ireland, UK
NTx	Osteomark® NTx Urine ELISA	nano-moles bone collagen equivalents/mL	Alere Inc, Waltham, MA, USA
<u>Neuro-inflammation and Afferent Neurotransmitters</u>			
NGF	Human β-NGF ELISA	2.3 – 5,000 pg/ml	RayBiotech, Norcross, GA, USA
BDNF	Human BDNF ELISA	26.4–16,000 pg/ml	RayBiotech, Norcross, GA, USA
CGRP	Human CGRP ELISA	1.0–100,000 pg/ml	Phoenix Pharmaceuticals Inc, Burlingame, CA, USA
Substance P	Substance P (Human, Rat, Mouse) ELISA	8.0–25,000 pg/ml	Phoenix Pharmaceuticals Inc, Burlingame, CA, USA
Collagenase I activity	Collagenase activity assays	U (cleavage of 1 mg collagen per min)/ml	Chondrex Inc. Redmond, WA

[‡]IL-interleukin, GM-CSF-granulocyte macrophage colony stimulating factor, TNF-Tumor necrosis factor, MMP-matrix metalloproteinase, NTx-amino-terminal telopeptide, crosslinked collagen, NGF-nerve growth factor, BDNF-brain-derived neurotrophic factor, CGRP-calcitonin gene related peptide. ELISA and activity assays were performed per manufacturers instructions with duplicate samples. The NGF ELISA kit from Promega (Promega NGF Emax ImmunoAssay System, Cat.# G7630, Promega, Madison WI) was not used as in 2014 it was taken off the market due to concerns regarding IgG cross-reactivity, (<http://onlinelibrary.wiley.com/doi/10.1002/nau.22882/full>). If duplicates varied by >10% CV, or if results exceeded the standard curve, assays were repeated either undiluted or, in the latter case, diluted 10-fold. If non-detectable by these standard curves, samples were assigned values of the lowest standard.

* Five candidate biomarkers were not considered in the final statistical analysis because urinary levels were below the sensitivity of the assay in >80% of samples: MMP-1 (detectable in 12%, cases; 11% controls), MMP-13 (19%, cases; 15%, controls), IL-1 β (16%, cases; 20%, controls), GM-CSF (10%, cases; 18%, controls) and TNF- α (1%, cases; 4%, controls).

Table 2

Summary of Demographic and Baseline Characteristics RUM Subjects (valid baseline samples)

Characteristic	Statistic/Category	Study Group		
		Active (N=260)	Control (N=54)	P-value
Age at Baseline (years)	N	260	54	0.85
	Mean (SD)	62.8 (11.9)	62.4 (16.2)	
	Median	64	62	
	Range	24, 89	24, 91	
Age Strata	<65	138 (53%)	28 (52%)	
	65	122 (47%)	26 (48%)	
Race	Am Ind/Alaskan Natl	3 (1%)	0	0.25
	Asian	0	1 (2%)	
	Black/African Am	27 (10%)	4 (7%)	
	White	221 (85%)	48 (89%)	
BMI (kg/m ²)	N	260	54	<0.001
	Mean (SD)	32.2 (8.2)	27.2 (5.7)	
	Median	31	26	
	Range	14, 75	18, 45	
Obese	No	122 (47%)	42 (78%)	<0.001
	Yes	138 (53%)	12 (22%)	
Current Smoker	No	228 (88%)	50 (93%)	0.16
	Yes	32 (12%)	3 (6%)	
Functional Comorbidity Index (FCI)	N	260	54	<0.001
	Mean (SD)	3.8 (2.3)	1.9 (1.9)	
	Median	4	1	
	Range	0, 10	0, 8	
Menopausal Status	Not sure	10 (4%)	0	0.006
	Postmenopausal	221 (85%)	39 (72%)	
	Premenopausal	29 (11%)	14 (26%)	
History Recurrent UTIs	No	226 (87%)	52 (96%)	0.02
	Yes	34 (13%)	1 (2%)	
Detrusor Overactivity	No	96 (37%)		
	Yes	164 (63%)		
OABq-SF Symptom Bother	N	259		
	Mean (SD)	74.9 (18.2)		
	Median	77		
	Range	27, 100		
OABq-SF HRQL	N	269		
	Mean (SD)	31.9 (18.8)		
	Median	31		

Characteristic	Statistic/Category	Study Group		
		Active (N=260)	Control (N=54)	P-value
	Range	0, 79		
UDI	N	260		
	Mean (SD)	59.7 (18.4)		
	Median	61		
	Range	0, 100		
IIQ	N	259		
	Mean (SD)	52.1 (27.2)		
	Median	52		
	Range	0, 100		
Sandvik	Slight	3 (1%)		
	Moderate	39 (15%)		
	Severe	69 (27%)		
	Very Severe	141 (54%)		

BMI: Body Mass Index; UTI: Urinary Tract Infection; OABq-SF: Overactive Bladder Questionnaire Short Form; HRQL: Health Related Quality of Life; UDI: Urogenital Distress Inventory; IIQ: Incontinence Impact Questionnaire. Of the 260 ROSETTA cases who provided baseline urine samples, 216 provide valid 3-month samples and 223 provided valid 6-month samples; of the 54 controls who provided baseline samples, 43 provided valid 3-month samples and 44 provided valid 6-month samples.

Table 3

Log Transformed Biomarker Data

Biomarkers	Baseline	3 Months		6 Months		P-value for Change over Time
		Onabotuli numtotoxin A N=108	Sacral Neuromodulation N=108	Onabotuli numtotoxin A N=113	Sacral Neuromodulation N=110	
Neuro-inflammation						
NGF (pg/mg Cr)						
Control	Geo Mean (SDLog)	5.0 (1.5)	10.4 (1.5)	9.4 (1.5)		0.031 ³
Case	Geo Mean (SDLog)	6.4 (1.5)	5.9 (1.5)	6.6 (1.5)	6.5 (1.6)	0.99 ¹ 0.38 ²
BDNF (pg/mg Cr)						
Control	Geo Mean (SDLog)	46.3 (1.2)	73.8 (1.3)	65.8 (1.3)		0.094 ³
Case	Geo Mean (SDLog)	63.0 (1.4)	56.5 (1.5)	47.5 (1.2)	58.5 (1.3)	0.51 ¹ 0.44 ²
Inflammation						
IL-6 (pg/mg Cr)						
Control	Geo Mean (SDLog)	3.0 (1.1)	3.4 (1.2)	3.3 (1.0)		0.96 ³
Case	Geo Mean (SDLog)	2.5 (1.5)	2.9 (1.4)	2.8 (1.4)	2.8 (1.4)	0.03 ¹ 0.26 ²
IL-8 (pg/mg Cr)						
Control	Geo Mean (SDLog)	37.2 (1.3)	45.9 (1.1)	48.8 (1.2)		0.502 ³
Case	Geo Mean (SDLog)	38.4 (1.1)	50.0 (1.1)	43.8 (1.1)	45.7 (1.1)	0.01 ¹ 0.002 ²
Afferent Pathways						
CGRP (pg/mg Cr)						
Control	Geo Mean (SDLog)	527.4 (1.5)	465.6 (1.4)	474.3 (1.5)		0.94 ³
Case	Geo Mean (SDLog)	595.5 (1.3)	563.3(1.5)	708.3(1.2)	656.0(1.2)	0.11 ¹ 0.16 ²
Substance P (pg/mg Cr)						
Control	Geo Mean (SDLog)	271.5 (1.1)	235.4 (1.1)	226.3 (1.3)		0.67 ³
Case	Geo Mean (SDLog)	257.5 (0.9)	252.5(1.0)	284.1(1.0)	264.3(1.0)	0.60 ¹ 0.29 ²
Matrix remodeling and proteases						
MMP-2 (pg/mg Cr)						

Biomarkers		Baseline	3 Months		6 Months		P-value for Change over Time
			Onabotuli numtotoxin A N=108	Sacral Neuromodulation N=108	Onabotuli numtotoxin A N=113	Sacral Neuromodulation N=110	
Control	Geo Mean (SDLog)	183.8 (1.5)		197.8 (1.4)		221.9 (1.4)	0.48 ³
Case	Geo Mean (SDLog)	251.8 (1.3)	212.7(1.4)	206.9(1.4)	231.1(1.4)	231.3(1.3)	0.94 ¹
MMP-9 (ng/mg Cr)							
Control	Geo Mean (SDLog)	28.8 (2.1)		34.3 (2.0)		36.1 (2.1)	0.70 ³
Case	Geo Mean (SDLog)	32.8 (1.9)	54.9 (1.9)	35.4 (1.9)	54.2 (1.7)	34.7 (2.0)	0.02 ¹
Tropoelastin ^{*1} (mg/mg Cr)							
Control	Geo Mean (SDLog)	9.6 (1.2)		13.7 (0.0)		13.5 (0.8)	0.14 ³
Case	Geo Mean (SDLog)	17.1 (0.9)	14.3 (0.9)	15.1 (0.9)	15.7 (0.9)	15.1 (1.0)	0.14 ¹
NTx ^{*2} (nM/mM Cr)							
Control	Geo Mean (SDLog)	15.6 (2.1)		34.5 (1.4)		39.0 (1.0)	0.02 ³
Case	Geo Mean (SDLog)	31.4 (1.3)	34.1 (1.2)	36.1 (1.2)	32.5 (1.4)	34.9 (1.0)	0.62 ¹
Collagenase (µg/min/mg Cr)							
Control	Geo Mean (SDLog)	138.8 (321.2)		58.8 (191.4)		63.3 (215.3)	0.14 ³
Case	Geo Mean (SDLog)	279.2 (449.0)	110.4 (263.5)	123.2 (299.5)	80.9 (206.3)	159.1 (402.9)	<0.001 ¹
							0.75 ²

¹ P-values represent comparison of change between baseline and six months in active case participants over time controlling for site and age strata;

² change from baseline in active participants between treatment groups controlling for site and age strata, and

³ change in mean biomarker level in control participants over time; with the exception of collagenase, all model-based analyses use natural log transformed values.

P-value in cases vs controls ***1**, **p=0.001**; ***2**, **p<0.001**; all other **p-values >0.05 between cases and controls**

Table 4

Multivariable Models

Parameter	Estimate ^I	95% CI	P-value
Model 1, Change in UUIE, Baseline Biomarkers			
Treatment	−0.46	−1.04, 0.12	0.12
Baseline UUIE	−0.56	−0.69, −0.43	<0.001
IL8	0.26	0.005, 0.52	0.049
CGRP	0.27	0.05, 0.50	0.017
Model 2, Change in OAB Symptom Bother, Baseline Biomarkers			
Treatment	−7.93	−25.6, 9.69	0.38
CGRP	3.98	1.12, 6.84	0.007
Substance P	4.33	−0.098, 8.076	0.056
NGF, Sacral Neuromodulation Treatment	−2.40	−6.20, 1.40	0.22
NGF, OnabotulinumtoxinA Treatment	5.03	1.40, 8.66	0.007
MMP-9, Sacral Neuromodulation Treatment	4.71	1.58, 7.84	0.004
MMP-9, OnabotulinumtoxinA Treatment	−0.57	−3.55, 2.40	0.71

^IThese values represent the parameter estimates for the covariates included in the model. For treatment, the parameter estimate represents the difference in change from baseline in the outcome variable (i.e. urgency urinary incontinence episodes (UUIE) or Overactive Bladder (OAB) Symptom bother) between baseline and six months between the two treatment arms. For the continuous variables (i.e. baseline UUIE or urine biomarkers), the parameters represent the slope in the regression relationship between the marker and the change in outcome. If the slope is positive then the change in the clinical outcome from baseline to six months becomes more positive (i.e. the clinical outcome degrades as negative changes represent improvement in both outcomes) as the level of the biomarker becomes larger.