

Richter H<sup>1</sup>, Moalli P<sup>2</sup>, Amundsen C<sup>3</sup>, Malykhina A<sup>4</sup>, Rogers R<sup>5</sup>, Myers D<sup>6</sup>, Paraiso M<sup>7</sup>, Albo M<sup>8</sup>, Nolen T<sup>9</sup>, Wallace D<sup>9</sup>, Meikle S<sup>10</sup>, Word A<sup>11</sup>

*1. University of Alabama at Birmingham, 2. University of Pittsburgh, 3. Duke University, 4. University of Colorado, Denver, 5. University of New Mexico, 6. Brown University, 7. Cleveland Clinic, 8. University of California, San Diego, 9. RTI International, 10. Eunice Kennedy Shriver National Institute of Child Health and Human Development, 11. University of Texas, Southwestern*

## THE ROSETTA URINARY MARKER STUDY

### Hypothesis / aims of study

The Refractory Overactive Bladder: Sacral NEuromodulation vs. BoTulinum Toxin Assessment (ROSETTA) trial randomized women with refractory urgency urinary incontinence (UUI) to sacral neuromodulation versus intradetrusor injection of onabotulinumtoxinA. The aims of RUM were to measure urinary biomarker levels in trial participants and controls at baseline, 3 and 6 months and to evaluate whether urinary biomarkers are related to UUI episodes (UUIE) and overactive bladder (OAB) symptom bother at 6 months.

### Study design, materials and methods

First morning urines were collected from trial participants and age-matched women without UUI. Of 16 candidate biomarkers, 11 were measurable in at least 80% of urine samples including those reflective of neuro-inflammation (NGF, BDNF), inflammation (IL-6, 8), afferent pathways (CGRP, Substance P) and matrix remodeling and proteases (MMP-2,-9, tropoelastin, NTx, collagenase activity). ELISA technology was used, except for collagenase (activity assay). Symptom bother was measured by Overactive Bladder Questionnaire.

### Results

Baseline and follow-up biomarkers levels are shown in the Table. Baseline biomarker levels differed little between cases and controls, except tropoelastin ( $p=0.001$ ) and NTx ( $p<0.001$ ). Biomarkers significantly changed post intervention included increases in IL-8 ( $p=0.005$ ) and MMP-9 ( $p=0.02$ ), both with greater increases in the onabotulinumtoxinA group ( $p=0.002$  and  $p=0.0002$ , respectively) and proportionate decreases in collagenase ( $p<0.0001$ ) in both treatment groups ( $p=0.75$ ). Multivariable analyses revealed: higher baseline IL-8 ( $p=0.049$ ) and CGRP ( $p=0.017$ ) independently associated with less reduction in UUIEs and higher CGRP ( $p=0.007$ ) with less reduction in OAB symptom bother at 6 months. Change in tropoelastin ( $p=0.046$ ) and CGRP ( $p=0.032$ ) levels over 6 months were significantly associated with UUIEs and OAB bother, respectively.

### Interpretation of results

Baseline tissue remodeling biomarkers were increased in cases. Increases in IL-8 and MMP-9 were sustained after intervention and greater in the onabotulinumtoxinA group. Conversely, collagenase decreased after both interventions. Baseline CGRP is predictive of 6-month outcomes. These data illustrate the complexity of refractory UUI and that matrix remodeling and neuropeptide mediation are likely involved in its pathophysiologic mechanisms and response to treatment.

### Disclosures

**Funding:** U10 HD041261 - Univ of Alabama at Birmingham; U10 HD069013 Brown Univ; U10 HD054214 Univ of California, San Diego; U10 HD054215 Cleveland Clinic; U10 HD041267 Duke Univ; U10 HD054241 Univ of Texas, Southwestern; U10 HD069025 Univ of New Mexico; U10 HD069006 Univ Of Pittsburgh; U10 HD069031 RTI International **Clinical Trial:** No **Subjects:** HUMAN **Ethics Committee:** Institutional Review Board **Helsinki:** Yes **Informed Consent:** Yes

Biomarker		Baseline	3 Months		6 Months		P-value
			Botox N=108	Interstim N=108	Botox N=113	Interstim N=110	
<b><u>Neuroinflammation</u></b>							
<b>Nerve Growth Factor (NGF) (pg/mg Cr)</b>							
Control	Geo Mean (SDLog)	5.0 (1.5)	10.4 (1.5)		9.4 (1.5)		0.988 <sup>1</sup>
Case	Geo Mean (SDLog)	6.4 (1.5)	5.9 (1.5)	6.6 (1.5)	6.5 (1.6)	6.5 (1.5)	0.382 <sup>2</sup>
<b>Brain-derived Neurotrophic Factor (BDNF) (pg/mg Cr)</b>							
Control	Geo Mean (SDLog)	46.3 (1.2)	73.8 (1.3)		65.8 (1.3)		0.372 <sup>1</sup>
Case	Geo Mean (SDLog)	63.0 (1.4)	56.5 (1.5)	47.5 (1.2)	58.5 (1.3)	59.5 (1.4)	0.437 <sup>2</sup>
<b><u>Inflammation</u></b>							
<b>Interleukin-6 (IL-6) (pg/mg Cr)</b>							
Control	Geo Mean (SDLog)	3.0 (1.1)	3.4 (1.2)		3.3 (1.0)		0.080 <sup>1</sup>
Case	Geo Mean (SDLog)	2.5 (1.5)	2.9 (1.4)	2.8 (1.4)	2.8 (1.4)	3.4 (1.5)	0.257 <sup>2</sup>
<b>IL-8 (pg/mg Cr)</b>							
Control	Geo Mean (SDLog)	37.2 (1.3)	45.9 (1.1)		48.8 (1.2)		<b>0.005<sup>1</sup></b>
Case	Geo Mean (SDLog)	38.4 (1.1)	50.0 (1.1)	43.8 (1.1)	45.7 (1.1)	44.7 (1.2)	<b>0.002<sup>2</sup></b>
<b><u>Afferent Pathways</u></b>							
<b>Calcitonin Gene-Related Peptide (CGRP) (pg/mg Cr)</b>							
Control	Geo Mean (SDLog)	527.4 (1.5)	465.6 (1.4)		474.3 (1.5)		0.282 <sup>1</sup>
Case	Geo Mean (SDLog)	595.5 (1.3)	563.3 (1.5)	708.3 (1.2)	656.0 (1.2)	730.5 (1.2)	0.165 <sup>2</sup>
<b>Substance P (pg/mg Cr)</b>							
Control	Geo Mean (SDLog)	271.5 (1.0)	235.4 (1.1)		226.3 (1.3)		0.788 <sup>1</sup>
Case	Geo Mean (SDLog)	257.5 (0.9)	252.5 (1.0)	284.1 (1.0)	264.3 (1.0)	276.1 (0.9)	0.294 <sup>2</sup>
<b><u>Matrix remodeling and proteases</u></b>							
<b>Matrix metalloproteinase-2 (MMP-2) (pg/mg Cr)</b>							
Control	Geo Mean (SDLog)	183.8 (1.5)	197.8 (1.4)		221.9 (1.4)		0.274 <sup>1</sup>
Case	Geo Mean (SDLog)	251.8 (1.3)	212.7 (1.4)	206.9 (1.4)	231.1 (1.4)	231.3 (1.3)	0.294 <sup>2</sup>
<b>MMP-9 (ng/mg Cr)</b>							
Control	Geo Mean (SDLog)	28.8 (2.1)	34.3 (2.0)		36.1 (2.1)		0.018 <sup>1</sup>
Case	Geo Mean (SDLog)	32.8 (1.9)	54.9 (1.9)	35.4 (1.9)	54.2 (1.7)	34.7 (2.0)	<b>&lt;0.001<sup>2</sup></b>
<b>Tropoelastin*<sup>1</sup> (mg/mg Cr)</b>							
Control	Geo Mean (SDLog)	9.6 (1.2)	13.7 (0.9)		13.5 (0.8)		0.169 <sup>1</sup>
Case	Geo Mean (SDLog)	17.1 (0.9)	14.3 (0.9)	15.1 (0.9)	15.7 (0.9)	15.1 (1.0)	0.102 <sup>2</sup>
<b>N-terminal telopeptide (NTx)*<sup>2</sup> (nM/mMcr)</b>							
Control	Geo Mean (SDLog)	15.6 (2.1)	34.5 (1.4)		39.0 (1.0)		0.768 <sup>1</sup>
Case	Geo Mean (SDLog)	31.4 (1.3)	34.1 (1.2)	36.1 (1.2)	32.5 (1.4)	34.9 (1.0)	0.818 <sup>2</sup>
<b>Collagenase (ug/min/mg Cr)</b>							
Control	Mean (SD)	138.8 (321.2)	58.8 (191.4)		63.3 (215.3)		<b>&lt;0.001<sup>1</sup></b>
Case	Mean (SD)	279.2 (449.0)	110.4 (263.5)	123.2 (299.5)	80.9 (206.3)	159.1 (402.9)	0.752 <sup>2</sup>

P-values represent comparison of <sup>1</sup>active case participants overtime controlling for site and age strata; and <sup>2</sup>change from baseline in active participants between treatment groups controlling for site and age strata; With the exception of collagenase, all model-based analyses use natural log transformed values. p-value in cases vs controls \*<sup>1</sup>, p=0.001; \*<sup>2</sup>, p<0.001.