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TITLE: Intravesical Lactobacillus to Reduce Urinary Symptoms after Spinal Cord Injury

PRINCIPAL INVESTIGATOR: Suzanne Groah, MD, MSPH

CONTRACTING ORGANIZATION: MedStar Health Research Institute, Washington, DC

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6. AUTHOR(S)
Suzanne Groah: Suzanne.l.groah@medstar.net

Inger Ljungberg: inger.h.ljungberg@medstar.net

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MedStar National Rehabilitation Hospital

102 Irving St, NW
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Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

N/a

14. ABSTRACT

Objective: This proposal specifically focuses on the population of ***people with SCI and neurogenic bladder who manage their bladders with intermittent catheterization (IC)***. The objectives of this study are to: 1) define clinically meaningful change (i.e., differentiating states of health and illness) with respect to urinary symptoms, urine inflammation, and the urinary tract's microbial ecosystem; 2) determine the optimal intravesical LactobacillusGG® (LGG®) dose to reduce urinary symptoms for use in a future clinical trial; and 3) advance diagnosis, self-management, and clinical research around urinary symptoms in SCI by developing a method for doing research that leverages computational methods, but focuses on integrating the patient's report of symptoms with gold standard (urinalysis and urine culture) and novel (urine NGAL and sequencing) clinical data
to reliably detect changes from "normal" that are clinically meaningful and interpretable-both *within and across* people.

Applicability: The short-term applicability of this work directly and immediately includes advancing: 1) *self-management* of symptoms with intravesical LGG®; and 2) *diagnostics* through differentiation of asymptomatic bacteriuria from urinary tract infection through use of uNGAL. The long-term applicability of this work includes advancing: 3) *prevention* of UTI with LGG®; and 4) reduction in over-prescription of antibiotics and resistant microbes.

Impact on Persons with SCI: This work specifically targets people with SCI who use intermittent catheters (due to neurogenic bladder). These individuals will potentially be helped by having an easily accessible method to reduce, through self-management, frequent and burdensome urinary symptoms, reducing the occurrence of *UTI and ultimately, antimicrobial resistance due to overuse of antimicrobials.*

Pilot Clinical Trial: The pilot clinical trial proposed in this project is a dose-finding experiment to determine whether "Low" (2 doses in 24 hours) or "High" (4 doses in 48 hours) dose self-instilled intravesical LGG® is superior, and which dose should therefore be used in a next-step clinical trial.

Project Timeline: To achieve a person-related outcome, we anticipate that it may take: 1) an additional 3-5 years beyond the completion of this trial to conduct the clinical trial to determine the efficacy of intravesical LGG®; 2) an additional 2-5 years for confirmatory assessment of uNGAL as a useful clinical marker, together with other outcomes we will study; and 3) an additional 3-5 years for definitive and reproducible testing of diagnostics models developed in this study. Of note, since LGG® is readily available (and does not require a physician's prescription) and this work represents a *new use* of this product (requiring an amendment to our existing Investigational New Drug approval from the FDA), once this study is completed, it will be available for immediate use by the SCI population for urinary symptoms while the efficacy clinical trial is ongoing.

Contributions of the Proposed Research to Advancing the Field, Patient Care and/or Quality of Life: Urinary tract infection is the most common outpatient infection world-wide, and for people with SCI, it is the most common infection, secondary condition, cause for emergency room visits, and infectious cause of hospitalization. Attempts to reduce UTI among people with SCI are stymied by long-standing diagnostic challenges which arise from evidence gaps around "gold standard" diagnostic tests (urinalysis and urine culture) that have lower sensitivity and specificity for UTI in SCI. A recent study of nearly 400 Veterans revealed that 87% had asymptomatic bacteriuria, and of these, 36% "were treated with antibiotics *unnecessarily*." Further, we have demonstrated in our pilot work that ***all*** of the people (in a national sample) who reported a history of UTIs had experienced at least one or more of 29 possible symptoms attributable to UTI during the previous year and that of the symptoms reported, the most common (***>80%)*** were cloudy, dark and/or bad smelling, urine. However, existing authoritative clinical guidelines specifically state that these same three symptoms are *not indicative of UTI*, in stark contradiction to the patient's perspective. This proposed research will incorporate the patient's perspective to advance our understanding around these very common and bothersome urinary symptoms. We will also determine whether urine NGAL can help to differentiate healthy from unhealthy urine in this population, advancing clinicians' ability to optimally care for their patients with SCI. Lastly, by refining our self-instilled intravesical LGG® intervention in preparation for efficacy testing in a future clinical trial, this work has the potential to improve quality of life by offering a self-management approach to reducing burdensome and common urinary symptoms.

15. SUBJECT TERMS None listed.					
16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT Unclassified	**b. ABSTRACT** Unclassified	**c. THIS PAGE** Unclassified	Unclassified	15	**19b. TELEPHONE NUMBER** *(include area code)*

TABLE OF CONTENTS

1. INTRODUCTION:

The long-term purposes of this research are to advance urinary symptom and urinary tract infection (UTI) evidence and antibiotic sparing therapeutics for urinary symptoms and UTI among the population of people with neurogenic lower urinary tract dysfunction due to spinal cord injury. The study population includes individuals with neurogenic bladder due to Spinal Cord Injury (SCI) over the age of 18 who utilize *intermittent catheterization for bladder management.* The objectives of the research among this population are: 1) to define clinically meaningful change (i.e. differentiating states of health and illness) with respect to urinary symptoms, urine inflammation, cultivable bacteria, and the urine ecosystem; and 2) to determine the optimal intravesical *Lactobacillus RhamnosusGG*® (LGG®) dose to be used to reduce urinary symptoms in a future clinical trial.

2. KEYWORDS:

Spinal cord injury, SCI, neurogenic bladder, neurogenic lower urinary tract dysfunction, NLUTD, urinary symptoms, bladder health, *Lactobacillus*, probiotics, paraplegia, tetraplegia

3. ACCOMPLISHMENTS:

After the initial delay due to the COVID-19 pandemic, we identified new procedures to safely collect urine samples while minimizing contact between study staff and study participants. In addition to that we developed procedures around safe specimen hand off between study staff at MedStar NRH and CNMC. At the time of this report we are successfully enrolling participants for both Aims.

What were the major goals of the project?

Sites: MNRN= MedStar National Rehabilitation Hospital; GU= Georgetown University; CNMC= Children's National Rehabilitation Hospital; UP= University of Pittsburgh

	Timeline	Complete	Date	Sites
Major Task 1: Create & prepare documentation for SA 1, and 2	Months			
Obtain FDA Amendment approval of current IND	Pre-award	100%	1/7/2020	MNRN
Refine eligibility criteria, exclusion criteria, screening protocol	1	100%	7/23/2019	MNRN, GU
Finalize consent form, protocol, high dose training manual	1	100%	7/23/2019	MNRN
Obtain IRB approval from MedStar IRB and CNMC IRB	2-3	100%	12/20/2019	MNRN, CNMC
Update Reliance Agreement between MedStar IRB and GU as needed		100%	Ongoing	MNRN, GU
Obtain Military 2nd level IRB review for all three sites (ORP/HRPO)	3-6	100%	5/19/2020	MNRN, CNMC, GU
Submit amendments, adverse events and protocol deviations as needed	As Needed	N/a	N/a	MNRN
Coordinate with sites for annual IRB report for continuing review	Annually	100%	Ongoing	MNRN
Maintain FDA IND active throughout study (Approved on: 07/10/2015)	1-36	100%	Ongoing	MNRN
Major Task 2: Coordinate Study Staff for Pilot Clinical Trials				
Subtask: Preparation for Pilot Clinical Trial				

Build and test REDCap Database	3-5	100%	5/1/202	CNMC
Order Supplies	5-6	100%	5/1/2020	MNRN, CNMC
Train Consumer Expert on Protocol	5-6	100%	6/5/2020	CNMC
Major Task 3: Participant Recruitment, Participant Evaluation (SA 1)				
Subject Screening and Enrollment	7-30	N/a	In progress	MNRN
Recruit 120 SCI participants from Washington, DC area	7-30	25%	In progress	MNRN
Collect first non-symptomatic urine specimens from each participant	7-30	25%	In progress	MNRN
Collect second non-symptomatic urine specimen from each participant	7-30	16.9%	In progress	MNRN
Prepare urine collections for assessment for urine NGAL, white blood cells, nitrite, cultivable bacteria, and the urinary microbiome	7-30	N/a	In progress	MNRN, CNMC, UP
Major Task 4: Participant Recruitment, Participant Evaluation (SA 2)				
Subject screening, enrollment, randomization, participant training	7-29	N/a	In progress	MNRN, GU
Recruit 182 SCI participants	7-30	37.3%	In progress	MNRN, GU
Randomize participants into low or high dosing arms	7-30	37.3%	In progress	GU
Send out study package to all participants including gloves, saline solution, lube, Lactobacillus GG capsules, and instillation guidelines	7-30	37.3%	In progress	MNRN
Of the 182, recruit n=68 local participants for urine pilot trial	7-30	38.2%	In progress	MNRN
Collect USQNB from participants weekly	7-30	N/a	In progress	MNRN
Collect before instillation urine samples from local sample (n=68) including 40% attrition rate	7-30	4.4%	In progress	MNRN
Collect post instillation urine samples from local sample (n=68) including 40% attrition rate	7-30	4.4%	In progress	MNRN
Prepare urine collection for and carry out UA, UC, and microbiome analyses	7-30	N/a	In progress	MNRN, CNMC, UP
Collect any unused Lactobacillus GG® capsules/ subjects used all Lactobacillus GG® capsules	8-31	10.1%	In progress	MNRN
Major Task 5: SA3 modeling activities				
Subtask 1: Model formation				
Obtain data from SA1 and SA2; data necessary for the modeling developed and validated in SA3 will be obtained in SA1 and SA2,or simulated based on these data and our preliminary data.	15	10%	In progress	GU
Analyze data from SA1 and SA2, integrating methods from data science and bioinformatics, using statistical model fitting to determine model utility.	15-36	10%	In progress	GU
Build and test a Bayesian Network using the combination of data science, bioinformatics, statistical model fitting, and clinical considerations, all applied to the data from SA1 and SA2, and/or simulated data based on the SA1 and SA2 results.		10%	In progress	GU
Generate probability estimates from a Bayesian Network that quantifies the likelihood that any given individual with the observed characteristics falls into each of the possible outcome categories.	2-36	10%	In progress	GU
Major Task 5: Dissemination of Study Results				

Prepare presentations for national and international dissemination	10-36	0%	Not yet started	MNRN, GU, CNMC, UP

What was accomplished under these goals?

Major Task 1: All documentation has been created and finalized for SA 1 and 2. Exempt IRB approval was received at Children's National Medical Center (CNMC) and the 2021 annual Continuation at MedStar IRB is currently under review. Our current approval is through November 16.2021. The ORP/HRPO submission was completed on January 27, 2020, and our submission was approved on May 19,2020. We continue to maintain our FDA IND #16306. All subtasks under Major Task 1 are either ongoing tasks or completed.

Major Task 2: All study related data is managed by REDCap at CNMC with weekly reports sent to study staff. Except for continuous training with Consumer Experts, all subtasks under Major Task 2 are completed.

Major Task 3: Enrollment of SA1 was delayed due to the COVID-19 pandemic. Protocols to minimize interaction with patients were developed and put in place. In addition, we developed a new drop off protocol for transferring samples to Children's National Medical Center (CNMC). CNMC has developed procedures for the use of lab space to minimize interaction for CNMC staff in the lab space. We have enrolled subjects for Aim 1 since August 2020.

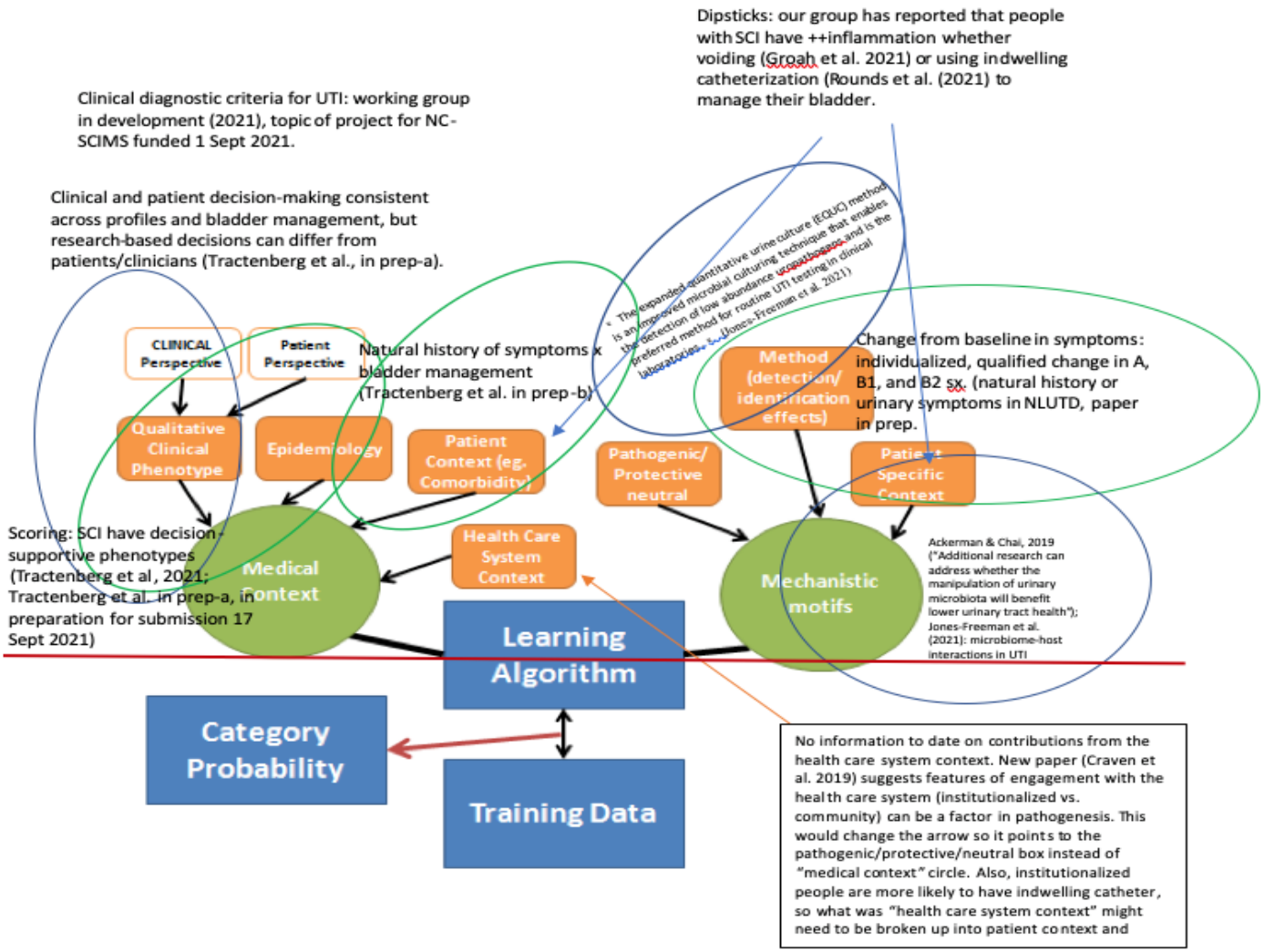
Major Task 4: Enrollment for SA2 began nationwide end of May 2020. Local enrollment begun in August 2020. As of August 14, 2021, 68 participants (26 of them local) have enrolled in the study to date (randomized into 35 high dose, and 33 low dose). All participants are sent supplies and receive training with the consumer expert prior to starting the study. 25 participants have completed the study so far.

Major Task 5:

Major Task 5: SA3 modeling activities	
Subtask 1: Model formation	
Obtain data from SA1 and SA2; data necessary for the modeling developed and validated in SA3 will be obtained in SA1 and SA2, or simulated based on these data and our preliminary data.	Data collection from SA1 and SA2 still in progress. Preliminary objective data for SA3 in revisions (as of 13 Sept 2021) (Groah et al 2021; Rounds et al. 2021) -see figure. Additional preliminary data from scoring paper (Tractenberg et al. 2021).
Analyze data from SA1 and SA2, integrating methods from data science and bioinformatics, using statistical model fitting to determine model utility.	Data collection from SA1 and SA2 in progress.

Build and test a Bayesian Network using the combination of data science, bioinformatics, statistical model fitting, and clinical considerations, all applied to the data from SA1 and SA2, and/or simulated data based on the SA1 and SA2 results.	Data collection from SA1 and SA2 in progress. Qualitative clinical phenotypes across three bladder management methods have been articulated in a manuscript in preparation for submission about 17 Sept 2021 (Tractenberg et al., 3 instruments paper).
Generate probability estimates from a Bayesian Network that quantifies the likelihood that any given individual with the observed characteristics falls into each of the possible outcome categories.	Not possible until SA1 & SA2 are complete and SA3 is much further along (e.g., months 30-36). New paper in preparation outlines the natural history of urinary symptoms across bladder management methods (Tractenberg in preparation).

Building and testing a Bayesian Network requires as-yet incomplete collection of the data from SA1 and SA2, even for simulated data based on the SA1 and SA2 results. Recent and new publications are contributing to our understanding of both what is known and what is totally unknown. The figure below shows the state of the literature in 2021, including three newly published papers by our research team (Groah et al. 2021; Rounds et al. 2021; Tractenberg et al. 2021), one paper about to be submitted for peer review (Tractenberg et al. in prep-a) and one paper that is in preparation (Tractenberg et al. in prep-b) that explores the natural history of urinary symptoms among people with NLUTD and SCI.



A clear gap in the literature is that there is no consensus on effective methods for longitudinal analysis of change in the microbiome. Recent and new publications are contributing to our understanding of both what is known and what is totally unknown, including Jones-Freeman et al. 2021 and Ackerman & Choi 2019. The lack of consensus makes it more difficult to extract mechanistic motifs, and more important to leverage existing data and results.

AckermanA L & Choi TC. The Bladder is Not Sterile: an Update on the Urinary Microbiome *Curr Bladder Dysfunct Rep*. 2019 ; 14(4): 331–341. doi:10.1007/s11884-019-00543-6.

Jones-Freeman B, Chonwerawong M, Marcelino VR, Deshpande AV, Forster SC, Starkey MR. The microbiome and host mucosal interactions in urinary tract diseases. *Mucosal Immunol* (2021). https://doi.org/10.1038/s41385-020-00372-5

Groah SL, Tractenberg RE, Rounds AK, Frost JK, Ljungberg IH. (In Press). Urinary Symptoms and Urine Dipstick Self-Assessment are unassociated in individuals with SCI, MS, and NLUTD who Void. *Topics in Spinal Cord Injury Research*.

Rounds AK, Tractenberg RE, Groah SL, Frost JK, Herminio N, Pham C, Ljungberg IH. (in press). Urinary Symptoms and Urine Dipstick Self-Assessment are unassociated in individuals SCI and NLUTD who Use an Indwelling Catheter. *The Journal of Spinal Cord Medicine.*

Tractenberg RE, Groah SL, Rounds AK, Davis L, Ljungberg I, Frost JK, Schladen MM. (2021). Clinical Profiles and symptom burden estimates to Support Decision-Making using the Urinary Symptom Questionnaire for People with Neurogenic Bladder (USQNB) using Intermittent Catheters. *Physical & Rehabilitation Medicine*. 2021 Mar;13(3):229-240. doi: 10.1002/pmrj.12479.

Tractenberg RE, Frost JK, Rounds AK, Yumoto F, Ljungberg I, Groah SL. (in prep-a). The spectrum of urinary symptoms among people with neurogenic lower urinary tract dysfunction (NLUTD).

Tractenberg RE, Frost JK, Yumoto F, Rounds AK, Ljungberg I, Groah SL. (in pre-b). Natural history of urinary symptoms among people with neurogenic lower urinary tract dysfunction (NLUTD): identifying “change from baseline”.

PROTOCOL (1 of 1 total):

Protocol [HRPO Assigned Number]: **E00733.1a**

Title: Healthy, asymptomatic state variability of urine in those with NLUTD (SA 1)
Target required for clinical significance: N=120

Protocol [HRPO Assigned Number]: **E00733.1a**

Title: Estimate the effect of intravesical LGG® 2 or 4 doses (SA 2)- Local sample with urine collection
Target required for clinical significance: N= 68

Protocol [HRPO Assigned Number]: **E00733.1a**

Title: Estimate the effect of intravesical LGG® 2 or 4 doses (SA 2)- Nationwide sample
Target required for clinical significance: N=114

SUBMITTED TO AND APPROVED BY: Application was submitted to HRPO and approval was obtained on May 19, 2020

STATUS:

			Enter information regarding number of subjects					
HRPO Protocol Number	**Protocol PI Name**	**Organization (Site)**	**# Target**	**# Enrolled**	**# Completed**	**# Screened**	**# Recruited**	**Other**
E00733.1a SA1	Groah	MedStar	120	30	22	346	30	Active
E00733.1a SA2 Local	Groah	MedStar	68	26	5	384	26	Active
E00733.1a SA2 Nationwide	Groah	MedStar	114	42	20	384	42	Active

What opportunities for training and professional development has the project provided?
Nothing to report

How were the results disseminated to communities of interest?
Nothing to report

What do you plan to do during the next reporting period to accomplish the goals?
During Year 3 of the grant, we will continue to enroll participants for SA1 and SA2 within the restrictions of our COVID-19 protocols. Protocols could be modified during the fall and winter should COVID-19+ cases increase in the Metropolitan Washington D.C area warranting changes in institutional policies and procedures involving research and/or face-to-face interactions. Planning to revisit recruitment criteria to possibly better target potential participants who will be more likely to meet inclusion criteria. Over the next year, interim data analysis will be completed, and abstracts will be submitted to nationwide conferences.

4. **IMPACT:** Nothing to report

What was the impact on other disciplines?

Nothing to report

What was the impact on technology transfer?

Nothing to report

What was the impact on society beyond science and technology?

Nothing to report

5. **CHANGES/PROBLEMS:**

Changes in approach and reasons for change

We have established protocols to minimize interaction between study staff and study participants due to COVID-19 pandemic. In addition, protocols have been established for delivery of urine specimen to CNMC staff and use of lab space at CNMC. We remain compliant with local government and

institutional policies regarding face-to-face interactions during the COVID-19 pandemic, and we will continue to do so if changes occur in future policies.

Actual or anticipated problems or delays and actions or plans to resolve them
Nothing to report

Changes that had a significant impact on expenditures
Expenditures during Year 2 of the grant are close to budgeted expenditure rate. Overall, we are still at a lower than anticipated expenditure rate due to not receiving study approval until May 2020, and the ongoing COVID-19 pandemic which is still limiting our enrollment efforts.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Nothing to report

Significant changes in use or care of human subjects
Nothing to report

Significant changes in use or care of vertebrate animals
Nothing to report

Significant changes in use of biohazards and/or select agents
Nothing to report

6. PRODUCTS:

Publications, conference papers, and presentations
Nothing to report

Journal publications.
Nothing to report

Books or other non-periodical, one-time publications.
Nothing to report
Other publications, conference papers and presentations.
Nothing to report

Website(s) or other Internet site(s)
Nothing to report

Technologies or techniques
Nothing to report

Inventions, patent applications, and/or licenses
Nothing to report

Other Products

No new materials to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name	Project Role	Research ID	% worked	Contribution to project
Suzanne Groah	Project PI	0000-0003-1213-1959	18%	No Change
Inger Ljungberg	Project Manager	N/a	8%	No Change

Amanda Rounds	Research Coordinator	0000-0003-0238-4629	51%	No Change
Kathaleen Brady	Research Coordinator	N/a	10%	No Change

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Yes. Dr. Groah (PI), and Dr. Tractenberg (Co-PI) have been awarded the Spinal Cord Injury Model System by NIDILRR. This is a 5 year grant and the funding period started 9/1/2021.

What other organizations were involved as partners?

Partner's contribution to the project: **Financial support; Collaboration**
Dr. Tractenberg is the Co-PI of the study. Her prime responsibilities completing the analysis of each aim. Dr. Tractenberg also designed, and will carry out, the modeling proposed for SA3.

Organization Name: University of Pittsburgh (Academic)
Location of Organization: Pittsburgh, PA
Partner's contribution to the project **Financial support; Collaboration**
Dr. Forster oversees all urine specimen study-related protocols including sample processing, freezing performed at Children's National Medical Center (CNMC). In addition. Dr. Forster will oversee uNGAL testing and preparation of microbiome libraries at University of Pittsburgh.

Provide the following information for each partnership:
Organization Name: Children's National Medical Center (CNMC)
Location of Organization: Washington, DC
Partner's contribution to the project **Financial support; Collaboration**
Study staff at CNMC developed the REDCap data management system used for SA1 and SA2. CNMC staff also receives all urine samples from the Clinical Research Coordinators and performs all preliminary specimen processing (i.e. pelleting, aliquoting, washing, and storage).

SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

This is reported in the SOW at the beginning of the document.

QUAD CHARTS:
See separate attachment

8. APPENDICES:

- Quad chart