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ARTICLE

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The association between urine neutrophil gelatinase-associated lipocalin and UTI in people with neurogenic lower urinary tract dysfunction

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Abstract

Study design Secondary analysis of urine samples collected from a prospective within-subject clinical trial.

Objectives Describe the baseline variation in urine neutrophil gelatinase-associated lipocalin (uNGAL) levels in adults with neurogenic lower urinary tract dysfunction (NLUTD) and determine if uNGAL levels vary according to likelihood of having a UTI.

Setting Greater Washington D.C. region.

Methods Urine samples were collected from a cohort of adults with NLUTD from a clinical trial. Samples were divided into groups of "Not UTI", "Unlikely UTI", and "Likely UTI" based on symptoms and urine culture results. uNGAL was compared between groups using Kruskal–Wallis and post hoc Dunn's test. Mixed effects logistic model was used to assess the association of uNGAL and Likely UTI.

Results Twenty-seven participants provided a total of 104 samples. uNGAL levels were lowest for the No UTI group ($n = 29$; 37 ng/ml interquartile range (IQR) (15, 71)), intermediate for the Unlikely UTI group ($n = 67$; 95 ng/ml IQR (37, 161)) and highest for the Likely UTI group ($n = 8$; 187 ng/ml IQR(146, 224)). uNGAL levels were higher in those with Likely UTI compared to both Unlikely UTI ($p < 0.05$) and No UTI ($p < 0.01$). uNGAL had an association with Likely UTI (OR 1.01, 95% CI (1.00–1.02), $p = 0.049$).

Conclusions Adults with NLUTD have notable variation in uNGAL levels in the absence of symptoms potentially due to UTI. uNGAL levels are higher in those who are likely to have UTI have higher uNGAL levels compared to those with non-specific symptoms and/or less growth on urine culture. uNGAL may have utility as a marker of UTI in people with NLUTD.

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Introduction

People with neurogenic lower urinary tract dysfunction (NLUTD) are at high risk for urinary tract infections (UTI) [1, 2]. However, accurately diagnosing UTIs in this population is challenging given the lack of a standardized and widely accepted definition. While the combination of a positive urine culture and pyuria in a symptomatic patient is sufficient for the diagnosis of UTI in people with normally functioning bladders, the same definition cannot be applied to those with NLUTD. People with NLUTD frequently have chronic inflammation of the urinary tract characterized by pyuria in the absence of symptoms, which is a primary reason that pyuria in the presence of symptoms is not a reliable biomarker and does not readily distinguish those

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with asymptomatic bacteriuria (ASB) from those with a UTI [3]. Other aspects of the routine urinalysis that are used to diagnose UTI in people with normally functioning bladders, such as nitrites, are frequently abnormal in people with NLUTD [4, 5] due to the pathogenic urobiome that characterizes the bladder of people with NLUTD [5–7]. Further, the presence of nitrites on the urinalysis are associated with unnecessary antibiotic use for UTI in people with spinal cord injury [8]. Finally, the use of symptoms to distinguish a UTI from ASB is challenging given the varying degree in sensation associated with various etiologies of NLUTD, as well as the frequency with which such symptoms occur [9].

Novel biomarkers have the potential to improve the ability to accurately diagnose UTIs in people with NLUTD. Urine neutrophil gelatinase-associated lipocalin (uNGAL) is a marker of acute kidney injury [10]. While uNGAL levels are usually within the normal range in patients with quiescent chronic kidney disease, which has been proposed as <9–54.5 ng/ml [11], elevations are seen in rapidly progressive forms of renal disease, such as in HIV-associated nephropathy [12, 13]. uNGAL is also a marker of UTI, and has utility in differentiating UTI from ASB in children with NLUTD largely due to spina bifida [14–17]. However, uNGAL has not been previously examined in adults with NLUTD, a large proportion of which are due to spinal cord injury or disease (SCI/D). Although prior work suggests that immune dysregulation may occur in the bladder of the spinal cord injured host, including a blunted uNGAL response to infection [18], there are no reports of uNGAL levels in adults with NLUTD in the literature. The objective of this study was to describe the baseline variation in uNGAL levels in a cohort of adults with NLUTD due to SCI/D, and to determine if a difference in uNGAL levels exists between people with no UTI, those unlikely to have a UTI, and those who are likely to have a UTI.

Methods

Study design

This is a secondary analysis of urine samples collected from an 18-month prospective, within-subject, three-stage clinical trial of an intravesical *Lactobacillus* intervention conducted in the United States (Clinical Trial NCT02748317). The phases of this study were baseline, intervention, and wash-out. The baseline and wash-out phases were observation-only phases. During the intervention phase, *Lactobacillus* was instilled as needed for specific combinations of urine symptoms as per study protocol. Participants' symptoms were monitored weekly for the duration of the trial using the Urinary Symptom Questionnaire for Neurogenic Bladder—Intermittent Catheterization version (USQNB-IC) [9].

Patients

People were eligible for inclusion in the study if they were at least 18 years of age with either spinal cord injury, multiple sclerosis, or spina bifida, used intermittent catheterization for bladder management, had at least two self-reported UTIs in the past year, and lived in the community setting. Patients with spinal cord injury had to be at least 1-year post-injury. Patients were excluded if they had genitourinary pathology other than NLUTD, were on either oral or intravesical prophylactic antibiotics, had a psychologic or psychiatric condition that precluded ability to follow directions, were pregnant or breastfeeding, had an immune deficiency or were on immune-modulating therapies, had taken an antibiotic within the two weeks prior to enrollment, or had a UTI within the two weeks prior to enrollment as defined by the Infectious Disease Society of America (IDSA) [3]. Any antibiotic exposure during the course of the study was recorded, but was not a reason for exclusion. This study was approved by the Institutional Review Board (#2014-211) and informed consent was obtained from all participants.

Urine samples

Urine samples were collected at various points throughout the study. These included points when the participant was in their baseline state of health without any symptoms, and at the onset of symptoms. During the intervention phase of the study, urine samples were collected before and within 48 h of *Lactobacillus* instillation. Although the post-instillation urine samples were not included in the overall analysis given potential of *Lactobacillus* to confound the results, a random sample of matched pre- and post-instillation pairs were analyzed to determine the effect of *Lactobacillus* on uNGAL levels.

Urine samples were collected by the participant using a new, unused catheter no more than 1 hour before delivery to the research team. Members of the research team drove to participants' homes to collect urine samples. Samples that were collected by the participant before the research team arrived were placed at 4° C immediately following collection. Aliquots of the urine were sent for urinalysis and urine culture at commercial clinical laboratories (Quest Laboratories). Within 6 hours of collection, the urine was centrifuged at 5000 RPM for 20 minutes. The supernatant, which was used in this work, was aliquoted and frozen at −80° C. Samples used in this work did not undergo any previous freeze-thaw cycles. Urine NGAL was measured by ELISA (Bioporto, Hellerup, Denmark). Urine NGAL values are not reported as normalized by urine creatinine given the varying muscle mass in people with NLUTD, which would potentially affect urine creatinine, but not uNGAL, levels.

Data collection

All patient-level data were obtained using REDCap [19] web-based surveys with automatic e-mail invitations. Participants' urinary symptoms were assessed using the USQNB-IC. The USQNB-IC is a list of 29 symptoms endorsed by people with, or by caretakers of people with, NLUTD [9, 20]. Participants completed these surveys weekly, or more frequently when symptoms developed, throughout the 18-months of the study.

Definitions

We categorized each urine sample into one of three groups according to increasing risk of UTI using the combination of clinical symptoms and bacterial growth on urine culture: No UTI; Unlikely UTI; and Likely UTI. The No UTI group included samples which had no growth on the urine culture at the same time that no symptoms were reported on the USQNB-IC. Symptoms were divided into those associated with Likely UTI and Unlikely UTI (Table 1). Urine cultures with growth of at least 10,000 CFU/ml of a single organism, a cut-off which is in accordance with the IDSA's guideline for treatment of catheter-associated UTIs [3], were considered positive. Samples in the Unlikely UTI group include both symptomatic samples that do not meet the Likely UTI criteria and asymptomatic samples with growth of at least 10,000 CFU/ml on urine culture (Table 1). Our definition of Likely UTI is similar to that proposed by the IDSA, although with a more stringent symptom requirement in order to better discriminate between those with UTI and those without.

Analysis

Standard descriptive statistics were used to report patient demographics. Urine white blood cells were reported as categorical, rather than continuous, variables and binned into groups based on the distribution of urine samples in each category. Chi-square, or Fisher's Exact, was used to compare the categorical variables between groups as appropriate. Given the small number of samples in the Likely UTI group, we first used a Kruskal–Wallis with a post hoc Dunn's test to compared uNGAL levels between groups despite the fact that patients gave multiple samples to determine if an overall difference existed between groups. We then used mixed effects logistic models to assess the predictive ability of uNGAL of predicting likely UTI with a random effect for patient to account for multiple assessments per patient. The dependent variable in all models was the dichotomous definition of Likely UTI vs all other groups. Given the small number of samples in the Likely UTI group, we were unable to add any additional covariates in the model. All analyses were done in R studio, version 1.1.447.

Results

Twenty-seven participants provided 104 samples, with a range of 1–8 samples per patient (Table 2). The participants in this study had a mean age at enrollment of 37.5 (standard deviation (SD) 11.2) years, were mostly male ($n = 19$, 70%), with spinal cord injury as the most common etiology of NLUTD ($n = 24$, 89%) (Table 3). There was one participant with spina bifida, and two with multiple sclerosis. The mean (SD) time since injury was 10.0 (9.4) years. All patients in the cohort had a history of UTIs, while a minority of patients had a history of bladder or kidney stones ($n = 8$, 22%, $n = 4$, 15%, respectively) or chronic kidney disease ($n = 3$, 11%) (Table 3).

We first looked at the difference in uNGAL levels in samples with differing degrees of pyuria. Median urine NGAL levels were higher in samples with either 11–40 urine white blood cells per high-power field (133 ng/ml, interquartile range (IQR) (90, 279) or >40 cells per high-powered field (176 ng/ml IQR (152, 240) compared to samples with ≤10 cells per high-power field (59 ng/ml IQR (22, 100) $p < 0.01$ for both comparisons). There was no difference in uNGAL between the groups with either 11–40 urine white blood cells and >40 urine white blood cells (Fig. 1).

We then evaluated the relationship between urine culture results and uNGAL levels among participants who were not experiencing symptoms ($N = 80$ samples from 27 participants). Of these, 26 samples had negative urine cultures, six had growth between 10,000 and 100,000 CFU/ml on urine culture, and 48 had growth of ≧100,000 CFU/ml on urine culture. The median uNGAL values among this group were: 37 ng/ml IQR: (15, 71) for samples with no growth on bacterial culture; 81 ng/ml, IQR: (63, 141) for bacterial growth between 10,000 and 100,000 CFU/ml; and 96 ng/ml IQR: (37, 169) for samples with ≧100,000 CFU/ml of bacterial growth on culture. The difference between the no growth and ≧100,000 CFU was statistically significant ($p < 0.01$), whereas there was no significant difference between the no growth and intermediate growth groups. We also used this subset of samples obtained during the absence of symptoms to examine the stability of uNGAL within the same participant. The data from these 13 participants, who provided 47 samples in the absence of symptoms, are shown in Supplementary Table 1.

We then looked at the samples stratified by likelihood of having a UTI using a combination of symptoms and culture results in the above-described groups of No UTI, Unlikely UTI, and Likely UTI. There were more samples with positive nitrites and more than 40 urine white blood cells in the Likely UTI group compared to the no UTI group (Table 4). Median uNGAL was higher in the Likely UTI group (187 ng/ml IQR(146, 224)) compared to both the No UTI (37 ng/ml IQR (15, 71), $p < 0.01$) and the Unlikely UTI

Table 1 Definitions of Likely UTI, Unlikely UTI, and No UTI.

Symptom classification	Actionable	Bladder	Urine quality	Aches and pains	Bowel and abdominal	Constitutional
Symptoms	• Feeling feverish • Increase in lower body tone, rigor, or spasticity • Increase in frequency or discomfort associated with bladder spasms • Increase in abdominal pain • Dizziness • Headache • Increase in irritability • Increase in fatigue, lethargy, or weakness	• Increased incontinence • Increased urgency • Increased leaking • Increased catheterization frequency • Pain with catheterization	• Cloudy urine • Foul-smelling urine • Dark urine	• Muscle aches • Increase in positional pain • Increase in pain in lower back • Increase in leg pain	• Nausea • Change in bowel patterns • Abdominal bloating	• Loss of appetite • Malaise • Altered sleep pattern
Sample classification	Symptoms					Urine culture
No UTI	No reported symptoms					No growth
Unlikely UTI	1) Any constitutional, aches and pains, bowel and abdominal, urine, or bladder symptom OR 2) Any actionable symptom alone or with any constitutional, aches and pain, urine, or bowel and abdominal symptom OR 3) No reported symptoms					≧10,000 CFU/ml
Likely UTI	At least one actionable symptom AND at least one bladder symptom					

Table 2 Number and distribution of samples provided by each participant.

Patient ID	Total samples ($n = 104$)	No UTI ($n = 29$)	Unlikely UTI ($n = 67$)	Likely UTI ($n = 8$)
1	3	0	3	0
2	1	1	0	0
5	1	1	0	0
6	2	2	0	0
7	3	0	3	0
8	2	0	2	0
10	3	0	3	0
13	4	2	2	0
16	1	1	0	0
20	4	4	0	0
28	6	0	5	1
41	4	1	1	2
42	1	0	1	0
51	4	0	4	0
57	3	0	2	1
64	6	0	2	4
73	7	2	5	0
75	5	3	2	0
76	8	1	7	0
77	7	1	6	0
78	7	1	6	0
80	6	2	4	0
81	5	3	2	0
87	3	1	2	0
92	1	0	1	0
102	4	2	2	0
103	3	1	2	0

Table 3 Patient demographics.

	All participants ($n = 27$)
Mean (SD) age (years)	37.5 (11.2)
Female	8 (30%)
Spinal cord injury	24 (89%)
Mean (SD) years since injury	10 (9.4)
History of UTI	27 (100%)
History of chronic kidney disease	3 (11%)
History of bladder stones	6 (22%)
History of renal stones	4 (15%)

All data expressed as: *n* (%) unless otherwise specified.

SD standard deviation.

group (95 ng/ml IQR (37, 161), $p < 0.05$). There was also a difference in uNGAL levels between No UTI and Unlikely UTI ($p < 0.01$) (Fig. 2). In the mixed effects logistic models, uNGAL did have a significant association with the presence

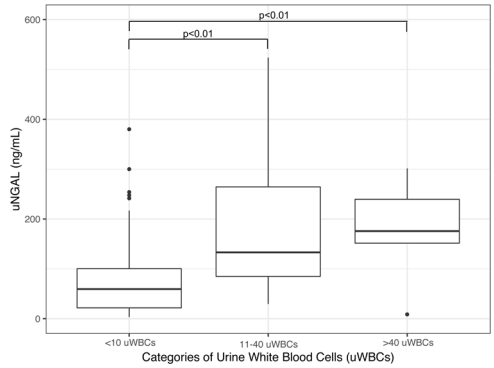

Fig. 1 Urine NGAL levels are elevated in samples with various numbers of urine white blood cells. Urine NGAL levels are elevated in samples with 11–40 urine white blood cells and >40 urine white blood cells compared to sample with between 0 and 10 urine white blood cells. uNGAL urine neutrophil gelatinase-associated lipocalin, uWBC urine white blood cell.

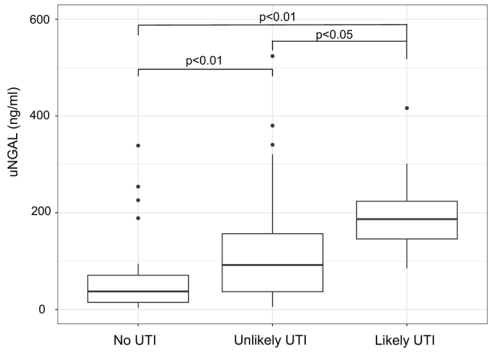

Fig. 2 Urine NGAL levels in samples with categorized as No UTI, Unlikely UTI, and Likely UTI. Urine NGAL levels are higher in samples categorized as Likely UTI compared to both samples categorized as Unlikely UTI and No UTI. Urine NGAL is also elevated in samples categorized as Unlikely UTI compared to No UTI. UTI urinary tract infection, NGAL neutrophi gelatinase-associated lipocalin.

Table 4 Urinalysis and urine culture results.

	No UTI ($n = 29$)	Unlikely UTI ($n = 67$)	Likely UTI ($n = 8$)	p values for pair-wise comparisons		
				No UTI vs Unlikely UTI	No UTI vs Likely UTI	Unlikely UTI vs Likely UTI
Nitrites	1 (3%)	41 (61%)	5 (63%)	<0.0001	0.0005	1.00
White blood cells categories						
0–10 cells/hpf	26 (90%)	42 (63%)	2 (25%)	0.007	0.0008	0.06
11–40 cells/hpf	3 (10%)	22 (33%)	3 (38%)	0.02	0.10	1.00
>40 cell/hpf	1 (3%)	3 (5%)	3 (38%)	0.55	0.007	0.01
Any growth on urine culture	0 (0%)	67 (100%)	8 (100%)	<0.001	<0.0001	1.00
Urine Culture with ≧100K CFU/ml	0 (0%)	59 (88%)	7 (88%)	<0.001	<0.001	1.00

All data presented as: n(%).

Hpf high-powered field, *CFU* colony-forming units.

of urinary symptoms indicative of UTI (odds ratio 1.01, 95% CI: (1.00–1.02), $p = 0.049$).

Finally, we performed a small sub-analysis of a random group of matched pre- and post-instillation urine samples. There was no significant difference between the median pre- and post-instillation uNGAL levels (197 ng/ml, IQR (108, 278), 173 ng/ml (369, 73), $p = 0.96$). The median difference between the post-instillation uNGAL and pre-instillation uNGAL was −5 ng/ml (IQR (−37 ng/ml, 98 ng/ml).

Discussion

Here, we provide preliminary evidence that uNGAL levels are associated with the likelihood of having a UTI when compared to those with a clinical profile of either Unlikely UTI or No UTI. We show that uNGAL tends to vary with the degree of pyuria and amount of bacteria on urine culture. However, neither pyuria nor urine culture results, together or in isolation, are sufficient to determine UTI risk in the NLUTD population. Our data demonstrate that uNGAL is clearly associated with clinical profiles of Likely UTI, suggesting its utility as a marker of UTI in people with NLUTD. Further, by including an intermediate risk group (Unlikely UTI), we present initial evidence of a dose–response relationship between the clinical and laboratory indicators of UTI and uNGAL levels, reinforcing uNGAL's potential utility as a biomarker of UTI in people with NLUTD.

Accurately diagnosing UTIs in people with NLUTD is difficult. This challenge lies in the incomplete understanding of how to define a UTI and interpret urinary symptoms and laboratory findings in this population. Indeed, there is no gold standard for the diagnosis of UTI in people with NLUTD. Given this significant gap in knowledge around UTI diagnosis in people with NLUTD, as well

as the risk of morbidity and mortality associated with untreated infections [21], there is a propensity to treat ASB in this patient population in the setting of diagnostic uncertainty [22]. Indeed, bacteriuria alone is associated with antibiotic use in people with spinal cord injury, even in the absence of symptoms [8]. Biomarkers may help clinicians determine when it is or is not appropriate to prescribe antibiotics for a UTI. Currently used biomarkers, such as urine white blood cells, are not sufficient to diagnose UTIs in people with NLUTD. This is emphasized in the IDSA guidelines for the management of catheter-associated UTIs, that the presence of pyuria is neither diagnostic of a UTI nor an indication for antibiotic treatment [3].

Urine NGAL is a promising biomarker of UTI that can be measured in the urine in 30 minutes using the ARCHITECT platform [23]. Given this rapid turn-around time, uNGAL has the potential to impact clinical management. However, as there are no current reports of uNGAL in the adult NLUTD population in the literature, it is first necessary to describe the levels of this biomarker in the absence of UTI. Urine NGAL levels have been described in a population of healthy adults, with a suggested reference range of <9–54.5 ng/ml [11]. Here, we describe large variations in uNGAL levels in our participants, many of which are beyond this reported normal reference range. In our cohort, only 42% of samples in the Unlikely UTI and No UTI group fell within this normal range. Further, the majority of the participants exhibit wide variability in uNGAL levels. One possible explanation is the presence of bacteriuria, as we report higher uNGAL levels in participants with positive urine cultures in the absence of symptoms compared to those with negative cultures. There are two reports of uNGAL in children with NLUTD, which both report elevated uNGAL levels in children with ASB, although not to the same degree as those with UTI, similar to the results reported here [16, 17]. As NGAL has a bacteriostatic effect in the urinary tract [24], an elevation in the setting of bacteriuria is consistent with its role in the local innate immune system [25, 26].

While bacteriuria is likely a large contributor to the variation in uNGAL levels, there are several individual participants with high uNGAL levels in the No UTI group. This suggests that other factors are also likely contributing to the variation in uNGAL levels. We evaluated whether the presence or number of urine white blood cells could be mediating this variation and found that participants with ≥10 urine white blood cells have elevated uNGAL levels compared to those with <10 urine white blood cells. However, there remains significant variation in uNGAL levels in samples grouped based on degree of pyuria, suggesting that this only accounts for a minority of the variation seen within uNGAL levels in our cohort. Another possible explanation could be related to changes in bladder dynamics in the setting of NLUTD, such as bladder wall ischemia in the setting of bladder spasticity, or bladder remodeling in the setting of over-distension. Indeed, a recent study of children with NLUTD reports a positive correlation between bladder compliance and uNGAL, further suggesting a relationship between bladder dynamics and uNGAL [27]. A final, although less likely, possibility is that a degree of chronic kidney disease could be affecting these results. Although prior work has shown that uNGAL is not elevated in quiescent kidney disease, elevations can be seen in more progressive forms of chronic kidney disease [12]. Our data suggest that the normal reference range for uNGAL in people with NLUTD is wider than that proposed for people with normally functioning bladders, due to both the frequency of bacteriuria and changes in bladder dynamics in this population. Despite this, in our cohort, uNGAL still differentiated people with NLUTD who we determined were likely to have UTI vs those who did not. However, before uNGAL is clinically implemented, further work is needed to better understand these variations and their implications for clinical care.

A major challenge associated with identifying markers of UTI in people with NLUTD is how to define UTI. One frequently cited definition is that proposed by the IDSA [3]. However, a challenge with operationalizing this definition is that the included symptoms are all weighted equally. Further, while many of these symptoms are present in UTI, they can also be attributed to other etiologies [9]. Therefore, while the IDSA's definition is sensitive, thus ensuring that treatment is not delayed in patients with potential UTI, it likely lacks a degree of specificity. The definition we used in this work includes a combination of symptom categories to increase the specificity of the diagnosis of UTI. We opted to use the more specific definition in this work, which in turn would allow for the identification of a more specific biomarker. As there is a trend of overtreatment of ASB in people with NLUTD [8, 22], a specific marker may help improve the appropriate use of antibiotics in this population.

There are several limitations to this study, including the small number of participants and the variation in the number of samples per participant. The small number of participants in the cohort limited our ability to assess the relationship between uNGAL and other variables, such as gender, pyuria, or bacterial burden. Further, we lacked data regarding variables such as adherence with oral medications, renal function or imaging at the time of symptoms to identify any potential renal involvement. The small number of samples associated with the outcome of interest, likely UTI, also limits the ability to fully model the effect of uNGAL while accounting for multiple samples per participant. Additionally, as the samples in this work were gathered as part of a separate study that involved intravesical *Lactobacillus*, there is a possibility that the *Lactobacillus* affected these results. However, as we excluded the

samples collected following the instillation, there is likely little effect of the *Lactobacillus* on the results in this work. NGAL is a real-time marker of both kidney injury and UTI. As such, levels fall rapidly following the initial stimulus, further decreasing the likelihood that the *Lactobacillus* affected uNGAL level [24]. We are further limited by the lack of a widely accepted definition of UTI in people with NLUTD, as well as an accurate way to clinically interpret urinary symptoms in this population. We used clinical profiles to identify people with symptoms most likely indicative of bladder pathology but realize that this may lead to misclassification of the samples. Finally, we did not normalize uNGAL to urine creatinine given the variable muscle mass and associated creatinine in this population.

Here, we demonstrate that people with NLUTD have a great degree of variation in uNGAL levels even in the context of a lack of symptoms and normal laboratory findings, although presence of bacteriuria is a likely contributor to some of this variation. Further, we show that uNGAL is elevated in people with NLUTD who are Likely to have UTI compared with those who are unlikely or definitely do not have UTI, an association that persisted when controlling for multiple samples per participant. These data, although preliminary, demonstrate that uNGAL is a promising urinary biomarker of UTI in people with NLUTD.

Data availability

The datasets generated and/or analyzed during the current study are available as Supplementary material.

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Author contributions CSF was responsible for study design, analyzing the data, interpreting the data, and wrote the first draft of the manuscript. OKL was responsible for running the assays, interpreting the data, and provided critical feedback on the manuscript. AR and IL consented the participants, collected the urine samples, collected symptom data, and provided critical feedback on the manuscript. BMS performed the first step of urine processing, collected the symptom data, provided data management, and provided critical feedback on the manuscript. SLG was responsible for study design, data interpretation, and provided critical feedback on the manuscript.

Compliance with ethical standards

Conflict of interest Bioporto provided partial salary support for SLG, IL, and OKL and provided the NGAL ELISAs for this work in kind. No other authors received support through Bioporto for this work. Bioporto did not play any role in the design of the study, collection, and analysis of data or the decision to publish.

Ethical approval This study was approved by the Institutional Review Board at MedStar National Rehabilitation Hospital. All participants underwent the informed consent process. We certify that all applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during the course of this research.

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