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## Predictive ability of NGAL in identifying urinary tract infection in children with neurogenic bladders

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### Abstract

**Background**—Distinguishing between urinary tract infection (UTI) and colonization (UTC) in patients with neurogenic bladders who require clean intermittent catheterization (CIC) is difficult. Urinary neutrophil gelatinase-associated lipocalin concentrations (uNGAL) are increased in UTIs. Our objective was to determine the predictive accuracy of uNGAL for UTI in CIC-dependent children.

**Methods**—Cross-sectional study of CIC-dependent patients from August, 2015 to November, 2016. UTI was defined as (1) growth of  $\geq 50,000$  cfu/mL of a uropathogen, (2)  $> 10$  urinary white blood cells/hpf, and (3) 2 of the following: temperature  $> 38$  °C, abdominal pain, back pain, worsened incontinence, pain with catheterization, or malodorous/cloudy urine. Positive urine cultures that did not meet these criteria were grouped as UTC, and negative cultures were grouped as no growth.

**Results**—Two hundred one patients were included (no growth = 100, UTC = 77, UTI = 24). Median (interquartile range) uNGAL was higher in the UTI group (UTI 1361 (931, 2516)  $\mu\text{g/g}$  creatinine, UTC 246 (106, 548)  $\mu\text{g/g}$  creatinine, no growth 36 (11, 179)  $\mu\text{g/g}$  creatinine,  $p < 0.01$  for all comparisons). The area under the ROC curve for uNGAL for UTI versus no UTI was 0.89, 95% CI (0.80–0.98).

**Conclusion**—uNGAL is elevated in CIC-dependent children with UTI compared to those with negative cultures and those with UTC.

### Keywords

Urinary tract infection; Neurogenic bladder; Clean intermittent catheterization; Pediatrics

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Compliance with ethical standards

**Conflicts of interest** Stuart Goldstein receives consulting fees and grant funding from Bioporto, Inc., which owns an assay for NGAL. No support from Bioporto, Inc., was used for the work reported in this manuscript.

The remainder of the authors do not have any conflicts of interest to disclose.

The study was approved by the Cincinnati Children's Hospital Medical Center Institutional Review Board with a waiver of informed consent as the urine samples were discarded specimens.

## Introduction

Children with neurogenic bladders who require clean intermittent catheterization (CIC) frequently have bacteriuria, which may be the result of a urinary tract infection (UTI) or bacterial colonization of the urinary tract (UTC) [1]. Distinguishing between these entities is important since UTI treatment prevents the spread of infection and resultant bladder and kidney damage, while unnecessary antibiotic exposure can lead to the development of antibiotic-resistant bacteria. However, establishing the diagnosis of UTI in children with neurogenic bladder who require CIC is difficult, as there are no standardized criteria to distinguish UTI from UTC. Due to this diagnostic uncertainty, and because of the risk for morbidity and mortality associated with an untreated infection, patients on CIC receive many courses of antibiotics for bacteriuria. As a result, the prevalence of antibiotic-resistant bacteria in this population is high, with multidrug resistant bacteria isolated from > 15% of urine cultures from CIC-dependent children, compared with 5% of urine cultures from children not receiving CIC [2, 3]. A method to identify patients who are at low risk for UTI, and therefore do not require antibiotics, is needed to prevent further increases in the rate of antibiotic resistance in this population.

Neutrophil gelatinase-associated lipocalin (NGAL) is a 24 kDa protein expressed and secreted by the genitourinary epithelium in response to a variety of injurious stimuli, including infection [4]. NGAL, which functions as part of the innate immune system in the genitourinary tract, is present in low levels in urine from healthy children and is increased in the setting of UTI [5, 6]. Therefore, we sought to measure urinary NGAL (uNGAL) concentrations in children with neurogenic bladders who require CIC, determine if uNGAL could discriminate between UTI and UTC in this patient population, and test the hypothesis that uNGAL levels could identify patients who are at low risk for UTI.

## Methods

### Patients

All patients with neurogenic bladder with CIC requirement followed at Cincinnati Children's Hospital Medical Center who had a urine culture sent as part of clinical care from August 1, 2015 through November 1, 2016 were eligible for inclusion in this cross-sectional study. Patients were identified in the electronic health record (EPIC™, Verona, WI) using the following International Classification of Diseases, 9th revision (ICD9), codes: neurogenic bladder (ICD9 596.54), spina bifida (ICD 741), paraplegia (ICD9 344.1), or quadriplegia (ICD9 344). The medical records of these patients were then manually reviewed to ensure that patients were actively performing CIC. Eligible patients were flagged in VigiLanz™ (VigiLanz Corporation, Minneapolis, Minnesota), a real-time lab monitoring software, which alerted the research team when a specified patient had a urinalysis or urine culture sent. Patients were excluded if there was evidence of acute kidney injury, defined per the KDIGO criteria [7], or sepsis [8] at the time of presentation, as both acute kidney injury and sepsis can increase NGAL concentrations [9], or if they had dialysis-dependent end-stage renal disease.

The study was approved by the Cincinnati Children's Hospital Medical Center Institutional Review Board with a waiver of informed consent as the urine samples were discarded specimens.

### Definitions

Patients were included in the UTI group if they met all three of the following criteria: (1) growth of greater than or equal to 50,000 colony forming units per milliliter (cfu/mL) of a single, known uropathogen from urine culture, (2) more than 10 urinary white blood cells/high-powered field in a spun urine specimen, and (3) two or more of the following signs and symptoms: fever greater than 38 °C, abdominal pain, new back pain, new or worsened incontinence, pain with catheterization, or malodorous or cloudy urine. This definition of UTI is adapted from that published by Madden-Fuentes et al., with a modified colony-count of 50,000 cfu/mL for UTI rather than 100,000 cfu/mL included in the initial definition [10]. Patients who did not meet these criteria, but had a positive urine culture, were included in the UTC group. Positive cultures without a concurrent urinalysis were excluded due to inability to categorize the culture. Patients with negative urine cultures were included in the no growth group. Cultures that grew fungi or an unidentified mixture of organisms were excluded from analysis.

### Clinical data

All clinical data were collected through manual chart review. The presence or absence of symptoms was taken from notes written by the primary clinician on day of sample collection. Symptoms for all patients with a positive culture were double-entered to ensure accuracy.

### Biomarker measurement

Residual urine that was sent to the laboratory as part of clinical care was obtained for processing within 10 hours of initial collection, centrifuged at 12,000 RPM for 10 min, and frozen at – 80 °C. The urine was refrigerated for a maximum of 10 hours between sample collection and processing. There were no freeze/thaw cycles prior to biomarker analysis. uNGAL was measured by ELISA (Bioporto, Grusbakken, Denmark), and urine creatinine by assay (Siemens Dimension RXL Max Clinical Analyzer, Munich, Germany) in the Division of Nephrology Biomarker Laboratory at Cincinnati Children's Hospital. Samples were frozen no more than 6 months prior to uNGAL and urine creatinine measurement. Analysis was completed with both the normalized (by urine creatinine) and non-normalized uNGAL values.

### Statistical analysis

Continuous variables were assessed for normal distribution, and nonparametric statistics were used for those with non-normal distributions. Normally distributed continuous variables (e.g., age) were compared using ANOVA with post-hoc Tukey for multiple comparisons. Urine NGAL was non-normally distributed, and therefore nonparametric testing was used to compare uNGAL concentrations between groups. The Mann-Whitney test was used when comparing two groups, and the Kruskal-Wallis test, with a post-hoc

Dunn test, was used for multiple groups. Categorical variables were compared with the chi-square test, or Fisher's exact as warranted. The area under the receiver operator characteristic (ROC) curve for identifying UTI was calculated using logistic regression models for uNGAL. The performance of uNGAL discriminating between UTI and UTC, and UTI and no UTI (UTC and no growth combined) was analyzed by the ROC curves. Each outcome (UTI vs UTC, UTI vs no growth, UTI vs no UTI) and each marker (normalized and non-normalized uNGAL) were modeled separately. The optimal uNGAL cut-point for the diagnosis of UTI, as well as the associated sensitivity and specificity, was determined using Youden's Index. Positive and negative predictive values, and positive and negative likelihood ratios, were calculated. Given the potential confounding effect associated with bladder augmentation in a subset of the enrolled patients, the analysis was completed both with and without patients who underwent bladder augmentation included in the cohort. All statistical analysis was done using R (version 3.2.5) [11] with package pROC [12]. A *p* value of < 0.05 was considered statistically significant. The datasets generated during the current study are available from the corresponding author on reasonable request.

We performed several subanalyses of this data given the lack of a gold-standard of UTI, which may have resulted in miscategorization of some cultures in this work. The first subanalysis used a stricter definition of UTI: we added the presence of nitrites (in the event of a gram-negative infection) to the definition of UTIs. In the second subanalysis, we excluded patients with positive cultures, positive nitrites, and no pyuria from the UTC group. In the third subanalysis, we excluded patients with positive cultures and positive nitrites from the UTC group.

## Results

Two hundred eighty-six cultures were reviewed, of which 85 (29.7%) were excluded (Fig. 1). Of the 201 cultures that were included, 100 were no growth, 77 were categorized as UTC, and 24 as UTI. Patient demographics and urinalysis comparisons are listed in Table 1. Patients in the UTC group were older and had a higher proportion of females compared with the no growth group. There was a higher proportion of patients with UTIs with anorectal malformation compared to those with no growth, but there were no other differences in the etiology of neurogenic bladder between groups.

Patients with UTI and UTC had a higher proportion of leukocyte esterase at all levels compared to patients with no growth, and there was a greater proportion of patients with greater than 50 urinary white blood cells in the UTI group compared with both UTC and no growth. The most common uropathogens in patients with UTC were *E. coli*, *Klebsiella pneumoniae*, and *Enterococcus*, while *E. coli* and *Klebsiella pneumoniae* were most common in patients with UTI. There was a higher proportion of patients in the UTI group who reported symptoms of abdominal pain, fever, flank or back pain, new or worsened incontinence, and cloudy or malodorous urine compared to those with no growth. There were more patients with abdominal pain, fever, flank, or back pain, and malodorous or cloudy urine in the UTI group compared to the UTC group. There was no difference in pain with CIC between all three groups (Table 1).

uNGAL normalized by urine creatinine (uNGAL/Cr) concentrations were higher in the UTI group (median, 1361  $\mu\text{g/g}$  creatinine; interquartile range (IQR) 931, 2516) than the UTC (246  $\mu\text{g/g}$  creatinine; IQR 106, 548) and no growth groups (36  $\mu\text{g/g}$  creatinine; IQR 11, 179,  $p < 0.01$  for all comparisons). Non-normalized uNGAL was also higher for those with UTI (434 ng/mL; IQR: 307, 969) than for those with UTC (135 ng/mL; IQR: 54, 224) or no growth (18 ng/mL; IQR 5, 78) ( $p < 0.01$  for all comparisons) (Fig. 2). The area under the ROC curve (AUC) for uNGAL/Cr for the diagnosis of UTI as opposed to no UTI was 0.89 (95% confidence interval 0.80–0.97). The AUC for non-normalized uNGAL for the diagnosis of UTI was 0.88 (95% CI 0.80–0.97). The AUCs for UTI using the stricter definition with the inclusion of nitrites were lower, although not significantly, than the AUCs for the definition without nitrites for all comparisons (Table 2). There were 22 patients with augmented bladders in this cohort (no growth = 5, UTC = 14, UTI = 3). After exclusion of these patients, there was an increase in the sensitivity (from 83 to 86%) and increase in the specificity (from 86 to 89%) of non-normalized uNGAL and associated changes in the likelihood ratios (Table 2). There were only minor differences in the other markers of predictive accuracy.

Using the definition without nitrites, in the entire cohort, including patients with augmented bladder, the cut-off for uNGAL/Cr was 817  $\mu\text{g/g}$  creatinine, with a sensitivity of 83%, specificity of 91%, positive predictive value of 58%, and negative predictive value of 97%. The cut-off for nonnormalized uNGAL was 229 ng/ml, with a sensitivity of 83%, specificity of 86%, positive predictive value of 47%, and negative predictive value of 97% (Table 2). Of the 163 samples with uNGAL/Cr less than the cut-off of 817  $\mu\text{g/g}$  creatinine, four (2.5%) were from patients with UTI. Similarly, there were only four patients (2.5%) with UTIs with non-normalized uNGAL less than the cut-off value of 229 ng/ mL. The results using the stricter definition of UTI, with the addition of nitrites, have higher sensitivity and lower specificity, with lower AUCs compared to the definition without nitrites (Table 2). When patients with UTC with positive nitrites are removed from analysis, as these patients may represent UTIs miscategorized as UTC, there are no major differences in the markers of predictive accuracy (Table 3).

Of the 101 total positive cultures, 14 grew gram-positive organisms, which had a wide range of uNGAL values. The one patient with a gram-positive UTI in our cohort had uNGAL values above both the normalized and nonnormalized cut-offs. At the cut-off value of 817  $\mu\text{g/g}$  creatinine, 12 of the 13 patients with gram-positive UTC had uNGALs below the cut-off. Conversely, at the cut-off value of 229 ng/ml, 12 of the 13 patients with gram-positive bacteriuria had uNGAL values below the cut-off.

## Discussion

In this study, we demonstrate that uNGAL is elevated in patients with neurogenic bladders who have UTI compared to those who have either UTC or no growth. Further, we show that uNGAL has a high negative predictive value for UTI in this population, which suggests that the potential use of this biomarker is to rule out UTI.

The most clinically relevant use of uNGAL in this population is to identify patients who are at low risk for UTI. Clinicians rarely need a marker to decide whether an ill-appearing patient with a likely source of infection requires antibiotics. However, a marker that can identify a patient as low risk for UTI may have utility when a febrile, but otherwise well-appearing, patient has bacteriuria. This is especially relevant in the outpatient setting, where clinicians are not able to easily observe patients off antibiotic therapy, which may influence the decision to initiate antibiotic treatment. Indeed, recent data demonstrates an increased prevalence of inappropriate antibiotic prescription in outpatient settings [13]. While patients with neurogenic bladders were not included in that prior study, the trend of inappropriate antibiotic prescription in the outpatient setting is likely also seen in patients with neurogenic bladder who present with concern for UTI. Our data suggests that uNGAL may be able to identify patients who are low risk for UTI who can be safely observed off of antibiotics.

The ability to determine when bacteriuria represents a UTI as opposed to UTC in children with neurogenic bladders at the point of care has important implications for this patient population. The frequency of UTIs with multidrug-resistant organisms seen in CIC-dependent children has been rising [2]. This increase in the prevalence of resistance is a result of multiple factors, including frequent exposure to antibiotics for either prevention or treatment of UTI [14]. Due to the difficulty in determining when bacteriuria represents UTC as opposed to UTI, as well as the consequences of an untreated UTI, these patients often receive antibiotics. Of the 51 patients in our cohort who received antibiotics at the point of care for presumed UTI, only 20 were categorized as UTI; the remaining 63% had no growth ( $n = 2$ ) or UTC ( $n = 30$ ). Of the four UTI patients who did not receive antibiotics at the point of care, three received antibiotics within 1 week following the culture. Of the 30 UTC patients who were given antibiotics (11 of whom had positive nitrites on urinalysis), the majority (90%) did not meet our definition of UTI based on lack of symptoms. The remaining 10% did not meet our definition due to lack of pyuria. Ten of these 30 patients were seen in an acute setting (e.g., Emergency Department) with concern for UTI, indicating that these patients may have been sicker at presentation, thus warranting antibiotics. The remaining 20 had urine cultures sent as part of routine urodynamics. Of these 20, ten were treated as the uropathogens were urease-producing organisms, and the remaining ten were treated due to presence of vesicoureteral reflux. Our data suggests that the use of normalized uNGAL at the point of care in for these patients may have avoided the prescription of antibiotics in 23 patients, suggesting that up to one half of antibiotics given at the point of care for presumed UTIs in CIC-dependent children may be unnecessary. The use of uNGAL at the point of care has potential to decrease the development of antibiotic-resistant infections in this population.

The utility of various parameters of the urinalysis to distinguish UTI from UTC is limited in patients who require CIC. The presence of pyuria, which is frequently used as a marker of UTI in the general pediatric population [15], lacks the same specificity in the neurogenic bladder population: CIC-dependent patients often have chronic inflammation of the genitourinary tract as a result of frequent catheterization [16], which confounds the utility of urinary white blood cells in this population. Further, bladder biopsy samples from children with myelomeningocele show abnormal epithelium with evidence of chronic inflammation [17], further limiting the utility of urinary white blood cells in the neurogenic bladder

population. Similarly, leukocyte esterase, another component of the urinalysis used clinically to diagnose UTI [18], is directly related to the presence of urinary WBCs, and therefore also has limited utility.

Nitrites are also an imperfect marker of UTI as the presence of nitrites is only indicative of the presence of specific organisms [19]. At baseline, the urine of patients with neurogenic bladder is home to bacteria that are classically considered to be pathogenic [20]. Therefore, the presence of nitrites on urinalysis only confirms the presence of a uropathogen, but is not indicative of an infection. Further, as it takes approximately 4 hours for bacteria to convert nitrates to nitrites, the amount of time that the urine is in the bladder also impacts the presence of nitrites on urinalysis [21]. Despite this, nitrites have good utility in predicting of bacteriuria in children with neurogenic bladder [22], and therefore are frequently used clinically in this situation. As 29% of patients in the UTC group had positive nitrites, we repeated the ROC curve analysis after excluding these patients as they may represent misclassified UTI patients. The measures of the predictive accuracy after the removal of the samples with nitrites have only minor differences in the normalized form of uNGAL. There are more notable differences in the non-normalized form, but the overall trends continue to have a high negative predictive value. Thus, while some misclassification may have occurred, as evidenced by the minor changes in the analysis when UTC patients with nitrites are removed, we believe that uNGAL remains a potentially useful approach to identifying patients in whom antibiotics may be safely withheld.

We report both the normalized and non-normalized uNGAL values in this work. Traditionally, uNGAL values have been normalized in research studies by urine creatinine to account for urinary concentration [9]. However, urine creatinine is an imperfect method of standardization for uNGAL in the setting of UTI. Urine creatinine comes from the filtrate at the level of the glomerulus, whereas uNGAL is produced by the collecting ducts [23], and is essentially free from glomerular filtration [24]. Similar to acute kidney injury, we show that uNGAL levels are increased in UTI regardless of whether or not NGAL is normalized [9], although the nonnormalized form of uNGAL has a greater sensitivity compared with the normalized form, which is more specific. Both forms have high negative predictive values, suggesting that the greatest utility of either form of uNGAL is to advise clinicians when antibiotics can be safely avoided. Further, uNGAL can be easily measured in a clinical laboratory without the need for creatinine normalization. In our institution, uNGAL results are reported within 2 h of receipt of the sample in our laboratory.

There has been limited work in the literature focusing on uNGAL in UTI. Several studies have shown that uNGAL is increased in UTI in the general pediatric population [6, 25], but to our knowledge this is the first work to describe the ability of uNGAL to distinguish between colonization and UTI in CIC-dependent children. While results from the general pediatric population are not directly comparable to those presented in this work due to the underlying differences in the genitourinary epithelium that is seen within the neurogenic bladder population, our results also show increased uNGAL concentrations in UTI.

There are several potential confounders to uNGAL concentrations in this cohort that are related to the complex physiology of this patient population. In addition to the genitourinary

epithelium, NGAL is also expressed by the gastrointestinal epithelium [26], and therefore patients with bladder augmentations may potentially have higher uNGAL levels than those without. However, our analysis shows that there are minimal differences in the predictive accuracy of uNGAL for UTI when patients with augmented bladders are included and excluded for the cohort, suggesting that the gastrointestinal epithelium is not a large contributor of uNGAL in this clinical setting. Other potential confounders include the possibility of obstruction, as uNGAL concentrations are elevated in the setting of acute urinary tract obstruction [27]. However, the increase in uNGAL concentrations in acute obstruction is likely due to acute kidney injury, which is not present in our cohort. Conversely, renal injury due to chronically increased bladder pressure results in chronic renal injury, and often occurs in conjunction with renal scarring due to associated vesicoureteral reflux [28]. Renal scarring has a doseresponse relationship with uNGAL concentrations, although the degree of increase is modest [29]. Therefore, while this may be confounding our results, the effect is limited, and likely does not affect the overall trends shown here. Finally, while patients with neurogenic bladders can develop increased pressure in the upper genitourinary tract, it is unclear which, if any, of the included patients had increased pressure and, if so, whether there were systematic differences among the groups. While much of the renal deterioration in this population begins in adolescence [30], our cohort was relatively young, suggesting that significant deterioration in renal function was less likely. Future work will focus on examining the relationship between biomarkers and changes in urodynamics in children with neurogenic bladder.

The other potential confounder is chronic kidney disease (CKD). While rapidly progressive forms of CKD are associated with significant increases in uNGAL concentrations, this is not seen in more quiescent forms of CKD that are classically seen in this population [31]. While CKD is present in a subset of our patients in this cohort, there is no difference in the proportion of patients with CKD between groups, suggesting that this is not a large confounder in our results. Finally, we have excluded patients with end-stage renal disease, as the effects of renal-replacement therapy on uNGAL concentrations is not well-described. Therefore, while CKD is present within a subset of patients in our cohort, it is unlikely to be significantly biasing these results.

Limitations of this work include the lack of a gold-standard method to diagnose UTI. As a result of the heterogeneity in criteria used to diagnose UTI, a standardized definition of UTI in the neurogenic bladder population was proposed for use in research [10]. We adapted this definition but chose to use a colony-count of 50,000 cfu/mL rather than the 100,000 cfu/mL in the initial definition, based on the American Academy of Pediatrics definition of UTI in which 50,000 cfu/mL is considered a clinically significant bacterial burden [15]. Further, we acknowledge that this is an imperfect definition, with inclusion of symptoms that not all clinicians would agree suggest UTI (e.g., cloudy or foul-smelling urine), chosen for consistency in this area of research. However, only 1 of our 30 UTI patients met criteria for UTI based on the symptoms of foul-smelling urine and fever; the remainder had additional symptomatology that allowed them to be classified as UTI. Therefore, while imperfect, this definition is acceptable for use in this study. A more sensitive definition of UTI should include an indication of clinical improvement following antibiotic treatment, or lack of progression of symptoms in patients who are not treated. Additionally, while reports



of symptoms were obtained through chart review, it is possible that pertinent positives were not recorded in the chart, thus leading to misclassification. This misclassification, which likely leads to the inaccurate classification of patients with UTI as UTC, may be responsible for the increased uNGAL concentration in the UTC group, compared to patients with no growth. Other limitations of this work include the lack of matching between groups. However, the differences in uNGAL levels between the genders and various ages is minimal, and thus likely would not affect our results [32]. Further, due to the small sample size of patients with UTI, we were unable to complete a sub-group analysis based on specific uropathogens.

## Conclusion

Here, we provide data suggesting that uNGAL has utility in discriminating between UTI and UTC in children with neurogenic bladders who require CIC. Urinary NGAL has a high negative predictive value for UTI, suggesting that a low concentration of uNGAL at the point of care may identify patients at low risk for the diagnosis of UTI in whom antibiotics can be safely avoided.

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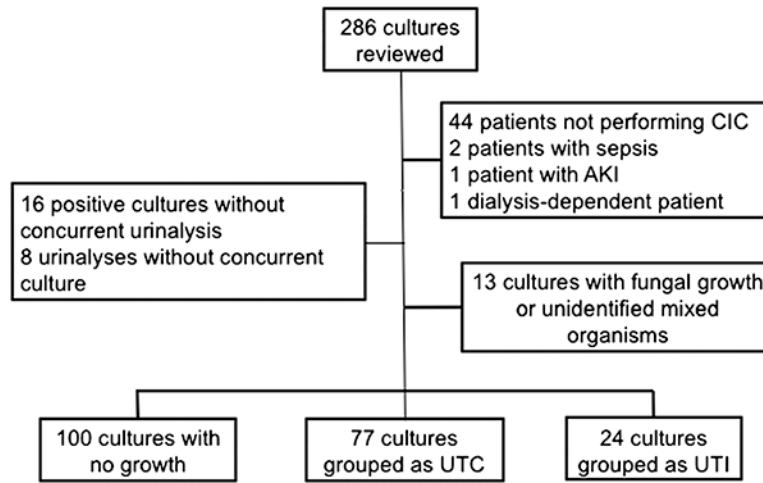
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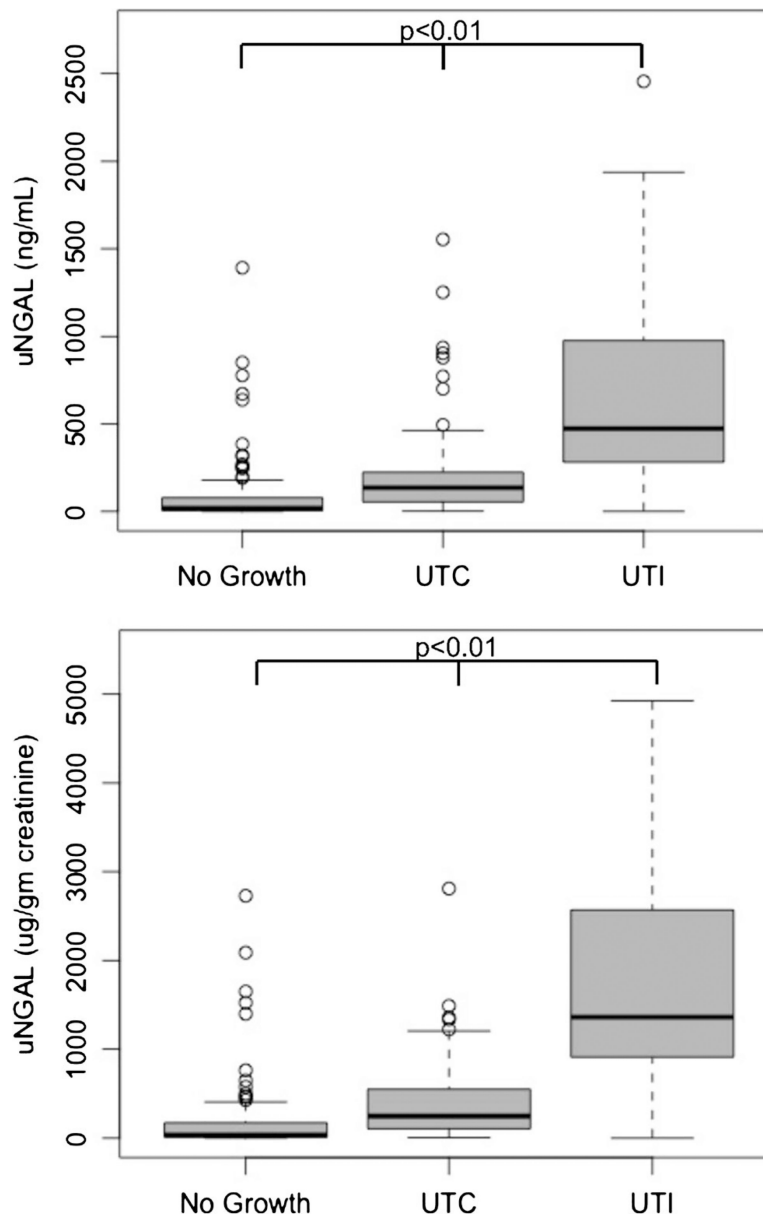
**Fig. 1.**  
Patient flow diagram

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**Fig. 2.** Boxplots of normalized and non-normalized uNGAL concentrations in “no growth,” “UTC,” and “UTI” groups. Normalized (top graph) and non-normalized (bottom graph) uNGAL concentrations in patients with no growth, UTC, and UTI. Significant differences exist between all groups for both the normalized and non-normalized uNGAL concentrations. *uNGAL* urine neutrophil gelatinase-associated lipocalin, *UTC* urinary tract colonization, *UTI* urinary tract infection

**Table 1**  
Demographics, urinalysis results, and uropathogens in patients with no growth, UTC, and UTI

	No growth (n = 100)	UTC (n = 77)	UTI (n = 24)
Age (years), mean (SD)	9.0 (6.4)	11.6 (7.1) <sup>a</sup>	8.6 (6.4)
Female	35 (35)	51 (66) <sup>a</sup>	13 (54)
Black	10 (10)	7 (9)	1 (8)
Hispanic	2 (2)	3 (4)	2 (8)
Fever on presentation	30 (30)	18 (23)	13 (54) <sup>a</sup>
Mitrofanoff	16 (16)	35 (46) <sup>a</sup>	7 (29)
Bladder augmentation	5 (5)	14 (18)	3 (13)
Chronic kidney disease	20 (20)	7 (9)	5 (21)
Etiology of neurogenic bladder			
Myelomeningocele	44 (44)	31 (41)	8 (33)
Anorectal malformation	9 (9)	12 (16)	5 (21)
Tethered cord	11 (11)	10 (13)	4 (17)
Urinalysis parameters			
Nitrites present	2 (3)	22 (29) <sup>a</sup>	10 (42) <sup>a</sup>
Absent LE	61 (75)	25 (32) <sup>a,b</sup>	0 (0) <sup>a</sup>
Trace LE	6 (7)	13 (17) <sup>a</sup>	1 (4)
Small LE	4 (5)	8 (10) <sup>a</sup>	3 (13) <sup>a</sup>
Moderate LE	4 (5)	13 (17) <sup>a</sup>	3 (13) <sup>a</sup>
Large LE	6 (7)	18 (23) <sup>a,b</sup>	17 (71) <sup>a</sup>
1–10 urinary WBCs	30 (56)	25 (35) <sup>a,b</sup>	0 (0) <sup>a</sup>
11–20 urinary WBCs	8 (15)	10 (13)	5 (21)
21–30 urinary WBCs	4 (7)	9 (12)	2 (8)
31–50 urinary WBCs	0 (0)	4 (6)	4 (13) <sup>a</sup>
> 50 urinary WBCs	4 (7)	17 (22) <sup>a,b</sup>	13 (54) <sup>a</sup>

	No growth (n = 100)	UTC (n = 77)	UTI (n = 24)
Uropathogens			
<i>E. coli</i>		36 (47)	13 (54)
<i>Klebsiella pneumoniae</i>		11 (14)	6 (25)
<i>Enterococcus</i> spp.		6 (8)	0 (0)
<i>Enterobacter</i> spp.		3 (4)	0 (0)
<i>Pseudomonas aeruginosa</i>		4 (5)	1 (4)
<i>Serratia</i> spp.		3 (4)	1 (4)
<i>Proteus mirabilis</i>		1 (1)	1 (4)
Symptoms			
Abdominal pain	7 (7)	10 (13) <sup>b</sup>	10 (42) <sup>a</sup>
Fever	6 (6)	13 (17) <sup>a,b</sup>	13 (54) <sup>a</sup>
Flank or back pain	3 (3)	6 (8) <sup>b</sup>	9 (38) <sup>a</sup>
New or worse incontinence	12 (12)	17 (22)	10 (42) <sup>a</sup>
Pain with CIC	8 (8)	4 (5)	2 (8)
Malodorous or cloudy urine	10 (10)	8 (10) <sup>b</sup>	10 (42) <sup>a</sup>

All data presented as n(%) unless otherwise stated

uNGAL, urine neutrophil gelatinase-associated lipocalin, UTC urinary tract colonization, UTI urinary tract infection, LE leukocyte esterase, WBC white blood cell, CIC clean intermittent catheterization

<sup>a</sup>  $p < 0.05$  compared with no growth

<sup>b</sup>  $p < 0.05$  compared with UTI

**Table 2**

Area under the ROC curve (AUC) for the diagnosis of UTI normalized and non-normalized uNGAL, clinical opinion, and nitrites, for all patients and all patients without augmented bladders

Predictor	AUC	Cut-off value	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Positive likelihood ratio	Negative likelihood ratio
Patients with augmented bladders included								
Diagnosis of UTI versus no UTI								
UTI definition does not include nitrites ( <i>n</i> with UTI = 24)	0.89 (0.80–0.98)	817	83	91	58	97	9.2	0.2
UTI definition does include nitrites ( <i>n</i> with UTI = 24)	0.88 (0.80–0.97)	229	83	86	47	97	5.9	0.2
UTI definition does include nitrites ( <i>n</i> with UTI = 11)	0.82 (0.65–1.0)	817	82	85	26	81	5.5	0.2
UTI definition does include nitrites ( <i>n</i> with UTI = 11)	0.84 (0.67–0.99)	228	91	81	23	99	4.8	0.1
Diagnosis of UTI versus UTC								
UTI definition does not include nitrites ( <i>n</i> with UTI = 24)	0.84 (0.73–0.95)	817	80	83	67	91	4.7	0.2
UTI definition does include nitrites ( <i>n</i> with UTI = 24)	0.83 (0.63–0.94)	229	80	83	67	91	4.7	0.2
UTI definition does include nitrites ( <i>n</i> with UTI = 11)	0.76 (0.57–0.94)	817	82	70	29	96	2.7	0.3
UTI definition does include nitrites ( <i>n</i> with UTI = 11)	0.79 (0.62–0.97)	229	91	72	33	98	3.3	0.1
Patients with augmented bladders excluded								
Diagnosis of UTI versus no UTI								
UTI definition does not include nitrites ( <i>n</i> with UTI = 21)	0.90 (0.80–1.0)	856	86	92	62	98	10.8	0.2
UTI definition does include nitrites ( <i>n</i> with UTI = 21)	0.89 (0.80–0.99)	229	86	89	54	98	7.8	0.2



Predictor	AUC	Cut-off value	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Positive likelihood ratio	Negative likelihood ratio
UTI definition does include nitrites ( <i>n</i> with UTI =10)	0.82 (0.63–1.0)	856	80	86	27	99	5.7	0.2
UTI definition does not include nitrites ( <i>n</i> with UTI = 21)	0.84 (0.65–1.0)	229	90	84	26	99	5.6	0.1
Diagnosis of UTI versus UTC								
UTI definition does not include nitrites ( <i>n</i> with UTI = 21)	0.85 (0.74–0.97)	856	81	85	49	96	5.4	0.2
UTI definition does include nitrites ( <i>n</i> with UTI = 10)	0.85 (0.74–0.96)	229	82	85	49	96	5.5	0.2
UTI definition does include nitrites ( <i>n</i> with UTI = 10)	0.75 (0.55–0.95)	1035	70	81	39	94	3.7	0.4
UTI definition does not include nitrites ( <i>n</i> with UTI = 10)	0.78 (0.59–0.97)	229	90	72	36	98	3.2	0.1

AUC area under the curve, ROC receiver operator characteristic, uNGAL urine neutrophil gelatinase-associated lipocalin, UTC urinary tract colonization, UTT urinary tract infection

**Table 3**

ROC Analysis with removal of UTC patients with positive nitrites and pyuria

Predictor	AUC	Cut-off value	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Positive likelihood ratio	Negative likelihood ratio
Diagnosis of UTI versus no UTI								
UTC with nitrites and no pyuria removed	0.92 (0.86–0.97)	817	86	89	49	98	7.8	0.2
All UTC with nitrites removed	0.92 (0.87–0.96)	229	91	84	41	99	5.7	0.1
Diagnosis of UTI versus UTC	0.92 (0.87–0.97)	816	86	90	56	98	8.6	0.2
UTC with nitrites and no pyuria removed	0.94 (0.81–0.97)	229	91	87	51	98	7.0	0.1
Diagnosis of UTI versus UTC	0.87 (0.79–0.96)	817	86	81	57	95	4.5	0.3
All UTC with nitrites removed	0.87 (0.79–0.95)	229	91	78	55	97	4.1	0.1
Diagnosis of UTI versus UTC	0.88 (0.80–0.97)	816	86	82	67	93	4.8	0.2
All UTC with nitrites removed	0.92 (0.85–0.98)	229	91	84	71	96	5.7	0.1

AUC area under the curve, ROC receiver operator characteristic, uNGAL urine neutrophil gelatinase-associated lipocalin