Urinary Neutrophil Gelatinase–Associated Lipocalin for the Diagnosis of Urinary Tract Infections

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abstract

OBJECTIVES: To determine the accuracy of the novel biomarker urinary neutrophil gelatinase–associated lipocalin (uNGAL) to diagnose urinary tract infections (UTIs) in febrile infants and young children.

METHODS: Prospective cross-sectional study of febrile infants <3 months (≥ 38.0°C) and children 3 to 24 months (≥ 39.0°C) evaluated for UTIs. uNGAL levels, urinalysis, Gram-stain and culture were obtained. UTI was defined by colony counts.

RESULTS: Of 260 patients, 35 (13.5%) had UTIs. Median uNGAL levels were 215.1 ng/mL (interquartile range: 100.3–917.8) and 4.4 ng/mL (interquartile range: 1.6–11.8) in the groups diagnosed with and without UTIs, respectively. The area under the receiver-operating characteristic curve for uNGAL was 0.978 (95% confidence interval [CI]: 0.948–1.000). At a threshold uNGAL level of 39.1 ng/mL, sensitivity was 97.1% (95% CI: 83.4–99.9) and specificity was 95.6% (95% CI: 91.7–97.7). uNGAL had higher sensitivity than the combination of leukocyte esterase (in trace or greater amounts) or nitrite (+) (97.1%, 95% CI: 83.4–99.9 vs 74.3%, 95% CI: 56.4–86.9), with similar specificity (95.6%, 95% CI: 91.7–97.7 vs 97.3%, 95% CI: 94.0–98.9). uNGAL had higher sensitivity than Gram-stain (97.1%, 95% CI: 83.4–99.9 vs 74.3%, 95%: CI: 56.4–86.9), with similar specificity (95.6%, 95% CI: 91.7–97.7 vs 100.0%, 95% CI: 97.9–100.0).

CONCLUSIONS: uNGAL has substantial accuracy to identify those with and without UTIs in infants and young children. Further studies will need to confirm our findings and determine if uNGAL is a more cost-effective test than standard screening tests.

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Dr Lubell conceptualized and designed the study, conducted the initial analyses, and drafted the initial manuscript; Dr Barasch assisted with the design of the study, provided laboratory support for sample analysis, and critically reviewed the manuscript; Ms Xu performed the laboratory analysis of study samples; Dr Ieni and Mr Cabrera coordinated and supervised data collection in the emergency department, reviewed the manuscript, and revised the manuscript; Dr Dayan conceptualized and designed the study, assisted with data analysis, and critically reviewed the manuscript; and all authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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WHAT'S KNOWN ON THIS SUBJECT: The standard urinalysis has shown variable accuracy to diagnose urinary tract infections (UTIs) in young children, leading to both under- and overtreatment. There is a need for biomarkers that are accurate and add benefit above existing tests.

WHAT THIS STUDY ADDS: Urinary neutrophil gelatinase–associated lipocalin (uNGAL) has substantial sensitivity and specificity to identify UTIs in febrile infants and young children. uNGAL is a novel biomarker with diagnostic accuracy potentially superior to urinalysis.

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Urinary tract infections (UTIs) are present in 5% to 10% of febrile children younger than 2 years.[1–3] Of the preliminary screening tests for UTI, urinalysis (UA) is the most convenient and readily available. However, a UA positive for leukocyte esterase (LE) or nitrites has shown variable and often suboptimal sensitivities (78%–88%) and specificities (72%–97%) across studies.[1,4–10] This variability likely reflects the differing definitions of UTI and methods of urine collection and laboratory procedures used to test the samples. Inaccuracy of the UA results leads to both the under- and overtreatment of infants with fever. There is a need for biomarkers that have more consistent test characteristics across sites, are independent of method of collection (not likely influenced by contaminants), and that identify true infection.

Studies in animals and humans reveal that neutrophil gelatinase-associated lipocalin (NGAL) may be an accurate marker for UTIs. NGAL is a protein expressed in neutrophils and several other human tissues, including α-intercalated cells in the collecting duct of the kidney.[11] NGAL is considered a component of the innate immune system and leads to inhibition of bacterial growth.[11] The urine and serum contain very low levels of NGAL protein at steady state, with expression of NGAL rising rapidly in response to cell damage caused by ischemia-reperfusion injury, presence of cytotoxins, and sepsis.[11–18] Urinary neutrophil gelatinase–associated lipocalin (uNGAL) is significantly elevated with Gram-negative UTIs in animal studies and studies in adults and children.[11,19–24] Given that upwards of 95% of UTIs are caused by Gram-negative bacteria such as *Escherichia coli*, assessing uNGAL levels may serve as a useful screening test for UTIs in children. Additionally, although neutrophils contribute to the uNGAL pool, the kidney intercalated cells are a predominant source of uNGAL.[11,25] In a rat UTI model devoid of neutrophils, uNGAL was still expressed.[25] Likewise, mice devoid of the kidney medullary cells responsible for uNGAL expression had markedly reduced uNGAL levels and impaired bacterial clearance of their UTIs,[11] supporting the role of uNGAL as a marker for UTIs independent of white blood cell activation (unlike LE or pyuria tests).

Although previous studies of uNGAL testing for the diagnosis of UTIs in children have revealed varying results (sensitivities: 70%–97%, specificities: 42%–83%),[21,22,24,26] the authors of a recent study noted excellent test characteristics in infants (sensitivity: 92.6%, specificity: 95.3%).[23] The variability across previous studies likely stems from differing methods of urine collection, NGAL processing, and the enrollment of children across broad age ranges, with differing criteria for urine testing. Further studies of uNGAL are necessary to understand its role and applicability to specific populations. Importantly, implementation of uNGAL use is feasible because analytical platforms are already approved for diagnostic use in Europe for acute kidney injury, delivering results in <1 hour.

Therefore, we aimed to determine the accuracy and test characteristics of uNGAL to detect UTIs in febrile children younger than 24 months of age. We also sought to compare the overall accuracy and test characteristics of uNGAL with the standard UA and Gram-stain for the diagnosis of UTIs.

METHODS

Study Design, Setting, and Participants

We conducted a prospective cross-sectional study of a convenience sample of febrile infants and children younger than 24 months for whom catheterized urine studies were obtained to evaluate for UTI, on the basis of clinician discretion. The study was performed at an urban tertiary care pediatric emergency department (ED) between August 2013 and October 2015. Children were eligible if they presented with fever (≥38.0°C for infants <3 months of age and ≥39.0°C for children 3 to 24 months of age) by any method, at home or in the ED, within the preceding 24 hours. Our goal was to include only otherwise healthy children. As such, we excluded children if they had a major congenital abnormality of any organ system, including but not limited to inborn errors of metabolism, congenital heart disease, any urogenital abnormalities (ie, hydronephrosis, vesicoureteral reflux, chronic renal disease, neurogenic bladder), chronic lung disease, or immune system disease. We also excluded patients for any of the following: received antibiotics within 48 hours of evaluation, presence of indwelling catheters or shunts, evidence of focal infections such as abscess or cellulitis, or definitive sources of fever, such as bacterial pneumonia, meningitis, varicella, or coxsackie virus.

To assess for enrollment bias and understand which patients were having urine obtained, we retrospectively reviewed 1 random day of every 2-week period during enrollment. We assessed whether patients enrolled were systematically different from otherwise eligible febrile children <24 months who (1) had urine obtained but were not enrolled or (2) had no urine obtained.

The local institutional review board approved the study with documentation of informed consent from the participant's legal guardian.

uNGAL and Standard Diagnostic Tests

For uNGAL testing, we collected up to 2 mL of urine via urethral

catheterization into a centrifuge tube. The urine was refrigerated immediately at 4°C. Within 12 hours of collection, the urine was centrifuged at 1000 rpm, and the supernatant was stored at −80.0°C. A minimum of 0.1 mL of urine was required for uNGAL processing. At the end of patient enrollment, we determined uNGAL concentration by enzyme-linked immunosorbent assays in a research laboratory. uNGAL was expressed in nanograms per milliliter by using a standardized commercial platform for total human NGAL (BioPorto KIT 036; Bioporto, Hellerup, Denmark). The laboratory technicians performing uNGAL testing were blinded to all clinical and laboratory data (eg, UA and culture results).

The UAs and Gram-stains were performed as per standard hospital laboratory procedure. Standard laboratory UA for nitrite and LE was completed by using the automated urine analyzer Iris iChem100 (Beckman Coulter Diagnostics, Brea, CA) on uncentrifuged urine. Point-of-care (POC) dipstick testing was performed by using the Siemens CLINITEK Status+ Analyzer. When both standard laboratory UA and POC dipstick testing were performed for the same patient, we used the standard laboratory UA in our analysis. Gram-stains were prepared by an automated system by using uncentrifuged urine and were read manually.

Reference Standard

Our reference standard was UTI determined by urine culture results, defined as follows: (1) a "definite" UTI as the growth of ≥100 000 colony forming units (CFUs)/mL of a single pathogen; (2) a "possible" UTI as the growth of 10 000 to 100 000 CFUs/mL of at least 1 pathogen, without contaminants; (3) a "contaminated" urine culture result as any growth of lactobacillus, micrococcus, diptheroids, *Bacillus* species or *Staphylococcus* epidermidis or any growth of 3 or more organisms; and (4) a urine culture with "negative" results: no growth or growth of <10 000 CFUs/mL of a single organism.[27]

Statistical Analysis

We analyzed patients for whom all tests were completed (uNGAL levels, UA, Gram-stain, and urine culture). Receiver-operating characteristic (ROC) curves were constructed to evaluate uNGAL's overall accuracy (via area under the curve [AUC]) in detecting UTIs by using SPSS software (IBM SPSS Statistics, IBM Corporation). We identified the optimal uNGAL-level threshold by manually assessing the tradeoff in sensitivities and specificities for all uNGAL levels. We identified the point on the ROC curve at which the tradeoff in sensitivity and specificity would maximize the AUC (ie, the point closest to 100% sensitivity and specificity). We subsequently calculated Youden's Index (J = Sensitivity + Specificity − 1) at and around this uNGAL-level threshold. The Youden's Index that is closest to 1 represents the optimized tradeoff in sensitivity and specificity. At the threshold value, we calculated 95% confidence intervals [CIs] for the uNGAL test sensitivity and specificity. We calculated the same test characteristics for the UA and Gram-stain as follows: (1) LE (trace or greater) or nitrite tests with positive results; (2) LE (trace or greater) and nitrite tests with positive results; and (3) any organisms present on a Gram-stain. Additionally, to compare our findings to previous findings in the literature on uNGAL, we assessed the test characteristics of uNGAL at a previously identified threshold of 20 ng/mL (at which sensitivity was 97% and specificity was 76%).[21]

For the primary analyses, we considered urine cultures as having positive results if their results met either the definite or possible criteria. Understanding that some possible UTI patients may not have had true UTIs, we repeated the analyses and treated possible urine cultures as having negative results. We conducted subgroup analyses for uNGAL results on the basis of patient age (0–3 and 3–24 months).

Given an ∼8% risk of UTI in the eligible population, which was based on the findings in previous literature,[2] we aimed to enroll up to 400 overall patients (32 with UTIs) in order to have the lower bound of the 95% CIs for the sensitivity of uNGAL to detect ∼80% of the UTIs (assuming 96% sensitivity at the uNGAL-level threshold value).

RESULTS

In Fig 1, we present a flow diagram of patients. Of the 260 patients analyzed, 35 (13.5%) had UTIs, of whom 22 (62.9%) had definite UTIs and 13 (37.1%) had possible UTIs; 32 of the 35 UTIs (91.4%) were caused by gram-negative organisms.

In Table 1, we note that the majority of the 84 children <3 months of age presented within 24 hours for evaluation of fever, compared with children 3 to 24 months of age, for whom the majority had urine obtained at least 24 hours after fever initiation. Overall, the children included in the study were well-appearing, with only 4.3% being described as ill.

In Table 2, we compare enrolled patients with eligible patients who were not enrolled. All febrile infants 0 to 3 months who were eligible but not enrolled (n = 22) had urine studies obtained as part of their evaluation. These infants were similar to those enrolled with respect to duration of fever and maximum temperature in the ED but had a lower rate of UTIs (not reaching statistical significance). For children 3 to 24 months, those who were not enrolled but had urine cultures

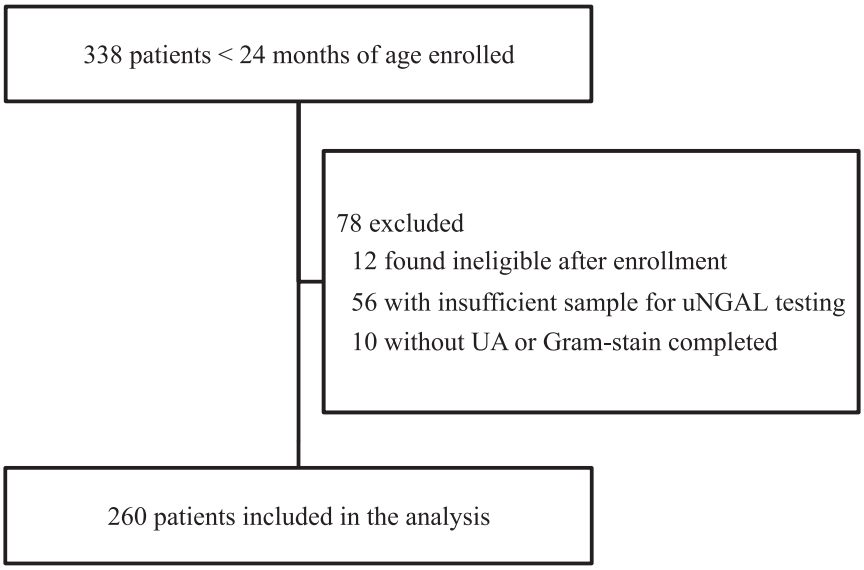

FIGURE 1
Flow diagram.

TABLE 1 Patient Characteristics and Clinical Findings

	All Patients, *N* = 260	0–3 mo, *n* = 84	3–24 mo, *n* = 176
Median age, d (IQR)	213 (68–416)	47 (27–66)	323 (207–474)
Boys, *n* (%)	118/260 (45.4)	50/84 (59.5)	68/176 (38.6)
Uncircumcised	78/118 (66.1)	32/50 (64.0)	46/68 (67.6)
Gestational age <37 wk, *n* (%)	23/260 (8.8)	5/84 (6.0)	18/176 (10.2)
Hispanic ethnicity, *n* (%)	207/247 (83.8)	62/82 (75.6)	145/165 (87.9)
History of previous UTI, *n* (%)	12/258 (4.7)	0/84 (0.0)	12/174 (6.9)
History of urologic abnormality, *n* (%)	5/256 (2.0)	2/84 (2.4)	3/172 (1.7)
Fever duration at home, *n* (%)			
<24 h	128/258 (49.6)	76/83 (91.6)	52/175 (29.7)
24–72 h	61/258 (23.6)	5/83 (6.0)	56/175 (32.0)
>72 h	69/258 (26.7)	2/83 (2.4)	67/175 (38.2)
Maximum temperature at home, °C, mean (SD)	39.4 (0.9)	38.6 (0.6)	39.7 (0.8)
Maximum temperature in ED, °C, mean (SD)	39.0 (1.0)	38.3 (0.7)	39.4 (1.0)
Ill appearance (YOS > 10), *n* (%)	11/253 (4.3)	3/83 (3.6)	8/170 (4.7)
Median WBC × 100/mm^3 (IQR)	12.0 (8–16)	11.2 (7.4–15.0)	12.3 (8.2 –17.3)

WBC, white blood cell; YOS, Yale Observation Scale.[28]

obtained (*n* = 49) were similar to those enrolled with respect to age, sex, and duration of fever but had a higher UTI rate (not reaching statistical significance). In contrast, those who were 3 to 24 months, and who were otherwise eligible but did not have urine studies obtained (*n* = 150), were older, predominantly boys, typically had another source of fever, and/or had a shorter duration of fever (<24 hours) compared with study patients.

In Fig 2, we show that median uNGAL levels were successively higher in those with no UTI (negative urine culture), a possible UTI, and a definite UTI. The median uNGAL concentration was 215.1 ng/mL (interquartile range [IQR]: 100.3–917.8) in the UTI group (definite and possible UTIs combined) and 4.4 ng/mL (IQR: 1.6–11.8) in the culture negative group. These differences persisted in both boys and girls, with a median uNGAL concentration of 4.5 ng/mL (IQR: 1.7–14.1) vs 4.4 ng/mL (IQR: 1.4–10.4) in the culture negative groups, 155.3 ng/mL (IQR: 49.6–228.5) vs 95.5 ng/mL (IQR: 47.6–173.3) in the possible UTI groups, and 278.1 ng/mL (IQR: 104.6–1102.3) vs 883.8 ng/mL (IQR: 353.2–1207.8) in the definite UTI groups, respectively.

In Fig 3, we display the ROC curve for uNGAL levels for the diagnosis of UTI. The AUC was 0.978 (95% CI: 0.948–1.000). At a threshold 39.1 ng/mL (the point at which Youden's Index was maximized [*J* = 0.927]), uNGAL testing had a sensitivity of 97.1% (95% CI: 83.4–99.9) and a specificity of 95.6% (95% CI: 91.7–97.7). The test characteristics of uNGAL at this threshold were slightly higher in patients 0 to 3 months (sensitivity of 100.0% [95% CI: 71.7–100.0], specificity of 97.2% [95% CI: 89.3–99.5]) compared with those 3 to 24 months of age (sensitivity of 95.5% [95% CI: 75.1–99.8], specificity of 94.8% [95% CI: 89.7–97.6]), although the 95% CIs were wide. At the uNGAL-level threshold of 20 ng/mL previously identified by Yilmaz et al,[21] uNGAL had a sensitivity of 97.1% and specificity 89.3%.

When we repeated the above analyses and considered possible UTIs as negative, the median uNGAL concentration was 622.6 ng/mL (IQR: 135.3–1142.4) in the UTI group (definite only) and 4.8 ng/mL (IQR: 1.7–14.3) in the group with negative results for UTI. The AUC for uNGAL

TABLE 2 Patients Enrolled Compared With Patients Eligible but Not Enrolled and Those Otherwise Eligible for Whom No Urine Was Obtained

	Enrolled 0–3 mo, $n = 84$	Not Enrolled 0–3 mo, $n = 22$	Enrolled 3–24 mo, $n = 176$	Not Enrolled 3–24 mo, $n = 199$	
	Urine Culture Obtained, $n = 22$		Urine Culture Not Obtained, $n = 150$	Urine Culture Obtained, $n = 49$
Age, d, median (IQR)	47 (27–66)	60 (45–71)	323 (207–474)	439 (327–569)*	333 (249–465)
Boys, *n* (%)	50/84 (59.5)	15/22 (68.2)	68/176 (38.6)	87/150 (58.0)*	19/49 (38.8)
Another source of fever[a], *n* (%)	36/83 (43.4)	5/22 (22.7)	92/174 (52.9)	118/150 (78.7)*	24/49 (49.0)
History of UTI, *n* (%)	0/84 (0.0)	0/22 (0.0)	12/174 (6.9)	1/150 (0.7)*	6/49 (12.2)
Fever duration at home, *n* (%)					
<24 h	76/83 (91.6)	20/22 (90.9)	52/175 (29.7)	72/150 (48.0)*	12/49 (24.5)
24–72 h	5/83 (6.0)	2/22 (9.1)	56/175 (32.0)	71/150 (47.3)*	22/49 (44.9)
>72 h	2/83 (2.4)	0/22 (0.0)	67/175 (38.2)	7/150 (4.7)*	15/49 (30.6)
Maximum temperature in ED, °C, mean (SD)	38.3 (0.7)	38.4 (0.4)	39.4 (1.0)	39.6 (0.4)*	39.8 (0.5)**
UTI (definite + possible), *n* (%)	13/84 (15.5)	1/22 (4.5)	22/176 (12.5)	—	10/49 (20.4)

—, not applicable.

[a] Another source of fever included upper respiratory tract infection, gastroenteritis, otitis media, or nonspecific viral syndrome.

* $P < .05$ comparing enrolled patients 3–24 mo with those 3–24 mo who were eligible but not enrolled and did not have urine cultures obtained.

** $P < .05$ comparing enrolled patients 3–24 mo with those 3–24 mo who were eligible but not enrolled and did have urine cultures obtained.

was 0.988 (95% CI: 0.977–0.999). At a threshold of 58.9 ng/mL, uNGAL had a sensitivity of 100.0% (95% CI: 81.5–100.0) and a specificity of 92.9% (95% CI: 88.6–95.7).

In Table 3, we compare the sensitivities and specificities of uNGAL to UA and Gram-stain. Of note, 251 patients had laboratory UAs performed; 9 patients had only POC UAs. uNGAL had higher sensitivity than UA, with similar specificity. Gram-stain had a somewhat lower sensitivity than uNGAL, but with high specificity. When we considered possible UTIs as negative, the sensitivity of LE increased to 86.4% (95% CI: 64.0–96.4). The sensitivity of LE was higher in the 0 to 3 month age group compared with the 3 to 24 month age group, with similar specificities (sensitivity of 84.6% [95% CI: 53.7–97.3], specificity of 95.8% [95% CI: 87.3–98.9] versus a sensitivity of 68.2% [95% CI: 45.1–85.3], specificity of 98.1% [95% CI: 94.0–99.5] (Supplemental Table 4). The AUC for a UA with LE or nitrite positive was 0.858 (95% CI: 0.770–0.946). The AUC for Gram-stain was 0.871 (95% CI: 0.784–0.959).

In Supplemental Table 5, we provide details of the 13 UTIs categorized as possible. Of the 13, 7 (53.8%) were caused by *E coli* growing at 10 000 to 100 000 CFUs/mL, with a median uNGAL concentration of 203.5 ng/mL. Both patients whose cultures grew a single gram-positive organism fell into the possible category (*Enterococcus faecalis* and Viridans group *Streptococcus*), with uNGAL levels of 40.2 ng/mL and 55.0 ng/mL, respectively.

DISCUSSION

In this prospective study, uNGAL had substantial sensitivity and specificity for use in identifying UTIs in infants and children younger than 24 months of age with fever. We identified a potentially optimal cutoff point for uNGAL levels to be 39.1 ng/mL

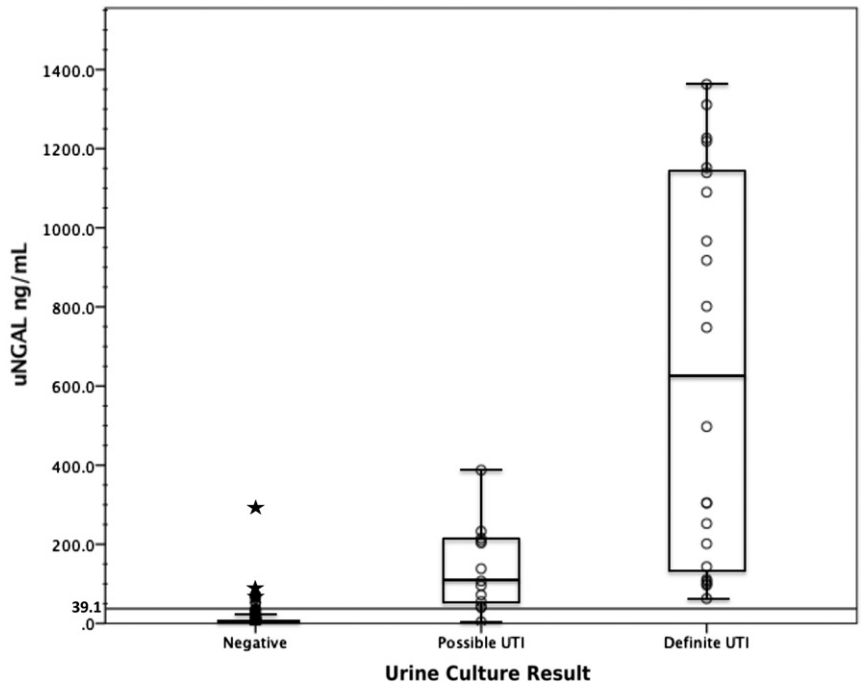

FIGURE 2

uNGAL levels in each UTI group. The scatter plot with box-and-whisker graph shows patient data points with boxes indicating the 25th and 75th percentiles, horizontal lines in each box indicating median values, whiskers marking the maximum and minimum values, and stars denoting outliers. Horizontal line represents the uNGALthreshold of 39.1 ng/mL.

in this population. Interestingly, median uNGAL levels were higher in definite as compared with possible UTIs. When compared with UA, uNGAL showed higher sensitivity and similar specificity, with overall higher accuracy (per AUC). Because the sensitivity of the UA was at the lower end when compared with its sensitivity in previous studies,[4–10] the benefit of uNGAL testing compared with UA requires further investigation.

The authors of previous studies of animals and humans have uniformly found that uNGAL levels rise significantly in response to UTIs, particularly Gram-negative UTIs.[11,17,19,20,29–31] Previous studies have also revealed that the expression of NGAL temporally correlates with the inciting stimulus (eg, bacteria), that it responds in a quantitative fashion to the bacterial load, and that it quickly reverses with resolution of infection.[11,13,17,19,32,33] These data and ours suggest that uNGAL has a role in the diagnosis of UTIs.

The authors of previous studies in which uNGAL was evaluated as a potential diagnostic tool for UTIs in children have noted varying test characteristics, although comparisons with our data are challenging because of the differing populations studied, definitions of UTIs, and assays used to determine uNGAL levels.[21,24,26] In a study of 60 children 2 months to 12 years of age, uNGAL levels were higher in the UTI group compared with controls (91.0 vs 14.3 ng/mL), with a sensitivity and specificity of 97.0% and 76.0% at a threshold of 20.0 ng/mL.[21] By contrast, a retrospective study of 107 children with and 337 children without UTIs (mean age of 4 years) revealed a uNGAL sensitivity of 70.0% (95% CI: 21.0–83.0) and a specificity of only 42.0% (95% CI: 36.0–89.0) at a threshold value of 5.8 ng/mL.[26] However, in this previous study, a permissive definition of UTI was used, and patients with asymptomatic bacteriuria and/or contaminants were likely included. In a recent case-control study of 108 infants with UTIs, the investigators identified an optimal uNGAL-level cutoff of 38 ng/mL (similar to ours), with a uNGAL sensitivity of 92.6% and a specificity 95.3%. Of note, 67% of urine samples in the UTI group and all urine samples in the control group were obtained by clean catch rather than by catheterization (as in our study).[23] This both lends support to the accuracy of uNGAL testing noted in our study and suggests potential use of uNGAL for less sterile circumstances such as clean catch.

The determination of whether uNGAL testing improves accuracy compared with UA has become complicated because the 2011 American Academy of Pediatrics guidelines for children suggest that the diagnosis of UTI requires pyuria.[27] However, these UTI guidelines do not include patients <2 months. Additionally, although most UTIs caused by *E coli* result in an inflammatory response (and positive UA results), several studies in children have revealed that certain non-*E coli* uropathogens and Gram-positive organisms are less likely to be associated with pyuria.[34–36] uNGAL tests may offer an advantage in this respect, because NGAL levels in UTIs appear to mainly reflect the kidneys' response to inflammation, independently of leukocyte activation.[11,25]

Interestingly, both infants and children in our study with UTIs caused by Gram-positive pathogens had increased levels of uNGAL (and UAs with negative results). The authors of previous studies in adults have also noted increased uNGAL levels with Gram-positive infections, although the levels were lower than with gram-negative infections.[19,20] Although NGAL has been shown to capture ligands produced by gram-positive organisms, the response may not be as robust as with

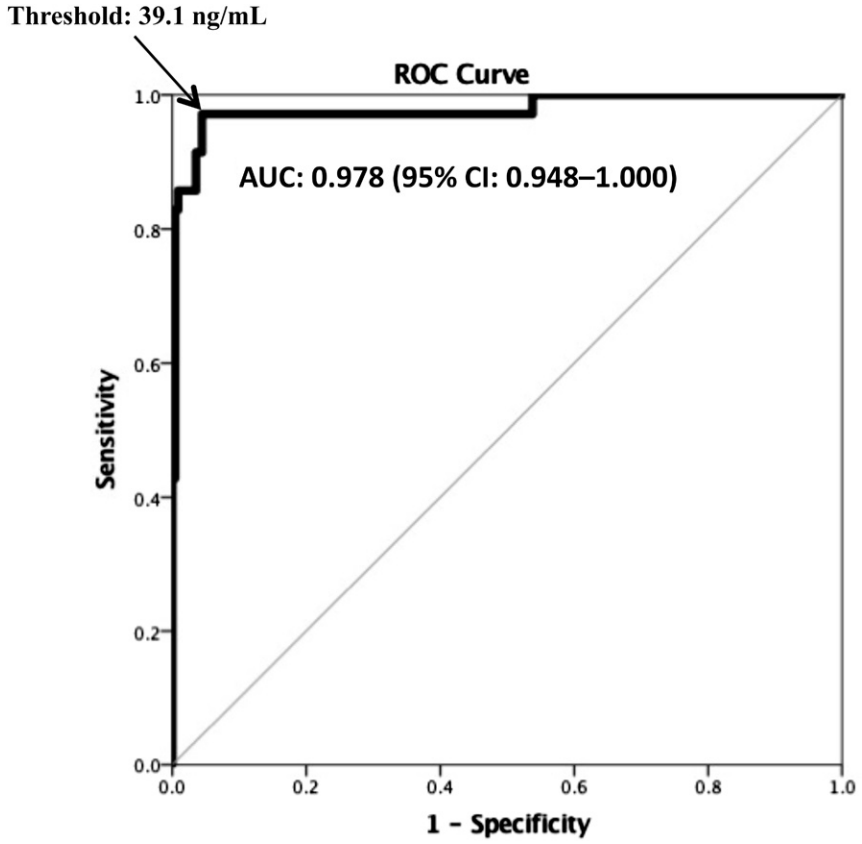

FIGURE 3
ROC curve for uNGAL for the diagnosis of UTI (definite and possible).

for infants and young children <24 months.[1–3] Moreover, in patients 3 to 24 months, we used a fever ≥39.0°C as an inclusion criterion because this temperature is used as a threshold in an internal guideline regarding the evaluation for UTIs.[3,38] It is unclear how our results would differ if we included children across a more broad temperature spectrum. Our sample was also predominantly Hispanic (84.0%), limiting the potential generalizability to other populations.

Furthermore, our laboratory reports included a 10 000 to 100 000 CFUs/mL group, which limited our ability to differentiate possible and definite UTIs. The lower sensitivity of UA in our study, compared with that in previous studies,[8–10] may reflect our definition of UTI, which combined possible and definite UTIs. The possible cases of UTI may have included patients with true UTIs, asymptomatic bacteriuria, and/or contaminants.

TABLE 3 Test Characteristics of uNGAL and Screening Urine Tests

Urine Test	Possible and Definite UTIs Combined (*N* = 260, 35 UTIs)		Definite UTIs (Possible Considered Negative) (*N* = 260, 22 UTIs)	
	Sensitivity % (95% CI)	Specificity % (95% CI)	Sensitivity % (95% CI)	Specificity % (95% CI)
uNGAL ≥39.1 ng/mL	97.1 (83.4–99.9)	95.6 (91.7–97.7)	100.0 (81.5–100.0)	90.8 (86.2–94.0)
UA				
Nitrite (+)	20.0 (9.1–37.5)	100.0 (97.9–100.0)	31.8 (14.7–54.9)	100.0 (98.0–100.0)
≥ Trace LE	74.3 (56.4–86.9)	97.3 (94.0–98.9)	86.4 (64.0–96.4)	94.5 (90.6–96.9)
LE ≥1+[a]	60.6 (42.2–76.6)	99.1 (96.4–99.8)	80.0 (55.7–93.4)	97.4 (94.2–98.9)
LE ≥2+[a]	60.6 (42.2–76.6)	100.0 (97.8–100.0)	80.0 (55.7–93.4)	98.3 (95.3–99.4)
LE ≥3+[a]	60.6 (42.2–76.6)	100.0 (97.8–100.0)	80.0 (55.7–93.4)	98.3 (95.3–99.4)
Nitrite (+) and ≥ trace LE	20.0 (9.1–37.5)	100.0 (97.9–100.0)	31.8 (14.7–54.9)	100.0 (98.0–100.0)
Either nitrite (+) or ≥ trace LE	74.3 (56.4–86.9)	97.3 (94.0–98.9)	86.4 (64.0–96.4)	94.5 (90.6–96.9)
Gram-stain	74.3 (56.4–86.9)	100.0 (97.9–100.0)	95.5 (75.1–99.8)	97.9 (94.9–99.2)

[a] Nine patients who only had POC dipstick testing completed were excluded from these analyses because POC LE tests were reported solely as having positive or negative results (rather than having trace results, 1+, 2+, or 3+).

gram-negative organisms because of the differing anatomic locations of the receptors in the kidney that sense these infections.[19,37]

Our study had several limitations. We studied a convenience sample of patients for whom physicians were obtaining urine studies to assess for UTI. Our surveillance for patients who were not enrolled clearly revealed that clinicians were using clinical criteria to determine which children 3 to 24 months required urine testing, with a rate of UTIs in our study population that was higher than that generally reported

The sensitivities of all preliminary urine screening tests, including uNGAL, may be underestimated if urine cultures have false-positive results caused by asymptomatic bacteriuria and/or contamination.

We did not attempt to distinguish upper from lower tract infections.

However, the authors of previous studies have suggested that 60% to 75% of young febrile children with bacteriuria will have evidence of acute pyelonephritis on imaging studies.[39] We also did not compare uNGAL testing with urine microscopy because microscopy was only performed either for UAs that were positive for LE or nitrite or when specifically ordered. However, the authors of previous studies suggest that UA (urine dipstick) compares favorably with microscopy in febrile infants and young children and may be an adequate stand-alone screen for UTIs.[4,5,8]

Additionally, we selected our uNGAL-level threshold post hoc to maximize accuracy. These results should be interpreted in the context of our small sample size and lack of a validation cohort. Finally, NGAL is an accurate marker for the identification of acute kidney injury.[31,40,41] Although we did not measure serum creatinine levels in the study participants, the included children were otherwise well, with no history of renal disease or neurogenic bladder, and would not be expected to have renal pathology that influenced uNGAL levels. The necessity to correct the uNGAL level for urinary creatinine level to account for urine osmolality is an area of controversy. However, the authors of previous studies have suggested that the correction of uNGAL levels for urinary creatinine did not significantly change the test characteristics when compared with uncorrected uNGAL levels.[23,42]

CONCLUSIONS

uNGAL levels have substantial sensitivity and specificity when used to identify those with and without UTIs in infants and young children. Further studies will need to both confirm our findings and determine the benefit and cost effectiveness of uNGAL testing compared with UA.

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ABBREVIATIONS

AUC: area under the curve
CFU: colony forming unit
CI: confidence interval
ED: emergency department
IQR: interquartile range
LE: leukocyte esterase
NGAL: neutrophil gelatinase–associated lipocalin
POC: point-of-care
ROC: receiver-operating characteristic
UA: urinalysis
uNGAL: urinary neutrophil gelatinase–associated lipocalin
UTI: urinary tract infection

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