Title: Urinary Tract Infections in Young Children: Testing a Novel, Non-Invasive, Point-of-Care Diagnostic Marker
Benjamin King, Pediatrics PGY-II
Mentor/PI: Tamar Lubell, MD

1. **Study Purpose and Rationale**

   Urinary tract infections (UTIs) are common in children, with more than 400,000 diagnosed annually in the United States. In children younger than 3 years, UTIs result in over 20,000 hospitalizations annually. Febrile children younger than 2 years have a particularly high risk and are frequently tested for UTIs, as UTIs are the most common serious bacterial infections in this age group. The timely diagnosis of UTIs is imperative, as delays in treatment are associated with substantial morbidity, including renal scarring.

   Unfortunately, the accurate assessment for UTIs in young children typically requires that they undergo bladder catheterization because the standard tests used to make accurate diagnoses depend on sterile urine collection. Bladder catheterization is painful to the child and distressing to the parent. Additionally, even when catheterization is completed, the standard urinalysis (UA) used to make a preliminary diagnosis has variable and suboptimal sensitivities (78-88%) and specificities (72-97%). Inaccuracy of the UA leads to both the under and over-treatment. There remains a substantial need for point-of-care (POC) biomarkers that have consistent accuracy to diagnose UTIs and do not require sterile urine and, therefore, do not require bladder catheterization.

   Our investigator team and others have shown that urinary neutrophil gelatinase-associated lipocalin (uNGAL), a biomarker already used to detect renal injury, is an extremely accurate marker for UTIs. In 260 children less than 2 years of age, we found that uNGAL had excellent sensitivity (97%) and specificity (96%) to identify those with and without UTIs. Moreover, studies in neonates suggest that uNGAL levels may not be affected by urine contamination, a potentially significant clinical advantage of uNGAL compared to the UA. No studies have compared uNGAL levels in bag specimens, which are frequently contaminated, to those from catheterized specimens, for the evaluation of UTIs. If uNGAL levels in bag specimens are comparable to those from catheterized specimens, children could have UTI evaluation completed by bag rather than by painful catheter urine collection. Furthermore, if an existing POC uNGAL test is more accurate than the standard POC UA, uNGAL may be used as a rapid test across clinical settings.

2. **Study Design and Statistical Procedures**

   We will perform a prospective cross-sectional study of febrile children 2-24 months being evaluated for UTIs. We will only include otherwise healthy children who have not received antibiotics within 48 hours of evaluation. The **outcome for our primary aim** is the **agreement** (measured as a proportion) between paired catheter and bag urinary uNGAL levels, analyzed separately for quantitative laboratory and semi-quantitative POC uNGAL testing methods. We base our sample size on the ability to have a narrow confidence interval around the estimate of agreement between paired catheter and bag uNGAL levels, dichotomized at a threshold of 39 ng/mL. Assuming an agreement of 95% between uNGAL levels in paired catheter and bag specimens, a **sample size of 400** will result in a 95% confidence interval of 92.4% to 96.9%. The **outcome for our secondary aim** is the **overall accuracy** of POC uNGAL and POC UA. We will measure accuracy via the area under the curve (AUC) and compare AUCs using non-parametric methods.

   Hypotheses

   1. Laboratory-based, quantitative uNGAL levels from paired catheter and bag urine specimens will show ≥ 95% agreement
   2. POC, semi-quantitative uNGAL levels from paired catheter and bag urine specimens will show ≥ 95% agreement
   3. POC uNGAL testing will have higher overall accuracy for diagnosing UTIs compared to POC UA from both catheter and bag urine specimens.

   Analyses for Primary Aim – Agreement in uNGAL levels between paired catheter and bag specimens**:**
   Hypothesis 1a (laboratory-based measurements): For the laboratory-based, quantitative uNGAL levels, we will conduct the main analysis by dichotomizing uNGAL, with ≥39 ng/mL being positive. At this threshold, we will estimate the proportion of agreement between catheter and bag specimens and its 95% confidence

interval. Additionally, we will separately determine the agreement for patients with and without UTIs. Although prior data suggest that uNGAL levels will not be substantially altered by urine concentration, we will conduct similar analyses of agreement in which we adjust the quantitative uNGAL for uCr, measuring the uNGAL/uCr ratio.

As secondary analyses, we will also examine quantitative uNGAL and uNGAL/uCr graphically in scatter plots, calculate Pearson's correlation coefficient, estimate the mean difference between paired specimens, and examine these mean differences using Bland-Altman plots. We would expect to see the largest variability between measurements at the higher end of quantitative uNGAL values. As such, we will also examine the coefficient of variation (ratio of standard deviation to the mean) for quantitative uNGAL measurements. A coefficient of variation will be calculated for each study subject. Summary statistics can then be provided to describe the coefficient of variation for the sample of subjects.

Hypothesis 1b (POC, semi-quantitative uNGAL levels): For POC, semi-quantitative uNGAL (at a threshold of 50 ng/mL), we will estimate the proportion of agreement between catheter and bag specimens and its 95% confidence interval. Using all of the semi-quantitative intervals (<20, 20-50, 50-150 and 150-300 ng/mL), we will also estimate Spearman's correlation and compare catheter vs. bag results using the Wilcoxon signed rank test.

Analyses for Secondary Aim – Accuracy of POC uNGAL compared to POC UA from catheter and bag specimens:

Semi-quantitative, POC uNGAL and POC UA test results will be assessed as binary variables (positive or negative). We define a POC uNGAL as positive if >50 ng/mL on the semi-quantitative color reading. POC UA will be defined as positive if either the LE test is positive (trace or greater) or the nitrite test is positive.

The reference standard will be the result of the urine culture, dichotomized as positive or negative. For the primary analysis, we will consider urine cultures positive if meeting either the "definite" or "possible" criteria (≥10,000 CFU's/mL). Understanding that some "possible" patients may not have true UTIs, we will repeat the analysis and treat "possible" urine cultures as "negative", using a stricter definition of UTI (≥100,000 CFUs/mL).

We will determine the accuracy of the POC tests (with 95% CIs) and compare the AUCs using non-parametric methods from correlated ROC curves detailed by DeLong, DeLong, and Clarke-Pearson.[38] We will compare the accuracies of POC uNGAL and POC UA from both a) catheter specimens and, b) bag specimens. Although AUCs describe the overall accuracy of a diagnostic test, we will also determine sensitivities and specificities for the POC tests (with 95% CIs) and compare the standard test characteristics using McNemar's test for paired samples.

3. Study Procedure

We will perform a prospective cross-sectional study of children 2-24 months of age evaluated for fever (≥ 38°C) in the Morgan Stanley Children's Hospital Pediatric Emergency Department (ED) and for whom catheterized urine studies are being obtained to evaluate for urinary tract infection (UTI), based on clinician discretion. Children will be eligible if they present with fever (≥38.0°C) by any method, at home or in the ED, within the preceding 24 hours.

4. Study Drugs

NA

5. Medical Device

NA

6. Study Questionnaires

NA

7. Study Subjects

Children will be eligible if they present with fever by any method, at home or in the ED, within the preceding 24 hours. Our goal is to include only otherwise healthy children. As such, we will exclude children if they have a major

congenital abnormality of any organ system including, but not limited to, inborn errors of metabolism, congenital heart disease, any urogenital abnormalities (i.e. hydronephrosis, vesicoureteral reflux, chronic renal disease, neurogenic bladder), chronic lung disease, or immune system disease. We will also exclude 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 including, but not limited to, bacterial pneumonia, meningitis, varicella or coxsackie virus. Finally, we exclude children younger than 2 months as practitioners routinely catheterize these young infants for urine culture, irrespective of urine screening test results, given the higher risk of invasive bacterial illnesses in this age group

8. **Recruitment of Subjects**
   Patients presenting to the ED for care will be enrolled in this study. A trained investigator, clinician, or research assistant will screen subjects for eligibility prior to approaching guardians for enrollment. The study will be discussed with the patient's guardian(s), and the guardian(s) of all eligible patients will be provided a study information sheet that provides study details and contact information. Once the guardian(s) are informed of the study details, written informed consent will be obtained for participation of the child. After the initial consent is obtained, guardians can choose to withdraw from the study at any time.

9. **Confidentiality of Study Data**
   Any endpoint devices used to maintain data will also be password-protected and encrypted. All patients will be assigned a unique identifier. Protected health information will not be reused or disclosed to any other person or entity, except as required by law or for authorized oversight of the research project. Only approved research staff will view the clinical information of children enrolled in this study. We will maintain the confidentiality of the data at all times, as dictated under HIPAA.

10. **Potential Conflict of Interests**
    NA
11. **Location of Study**
    Pediatric Emergency Department of MSCHONY
12. **Potential Risks**
    NA
13. **Potential Benefits**
    NA

14. **Alternative Therapies**
    NA

15. **Compensation to Subjects**
    NA

16. **Costs to Subjects**
    NA

17. **Radiation or Radioactive Substances**
    NA

Literature References:

1. Freedman AL. Urologic diseases in North America Project: trends in resource utilization for urinary tract infections in children. *J Urol*. 2005;173(3):949-954.
2. Hoberman A, Chao HP, Keller DM, et al. Prevalence of urinary tract infection in febrile infants. *J Pediatr*. 1993;123:17-23.

3. Hoberman A, Charron M, Hickey RW, et al. Imaging studies after a first febrile urinary tract infection in young children. *N Engl J Med*. 2003;348(3):195-202.
4. Shaikh N, Mattoo TK, Keren R, et al. Early Antibiotic Treatment for Pediatric Febrile Urinary Tract Infection and Renal Scarring. *JAMA Pediatr*. 2016;170(9):848-854.
5. Jacobson SH, Eklöf O, Lins LE, et al. Long-term prognosis of post-infectious renal scarring in relation to radiological findings in childhood--a 27-year follow-up. *Pediatr Nephrol*. 1992;6(1):19-24.
6. Shaw KN, McGowan KL, Gorelick MH, et al. Screening for urinary tract infection in infants in the emergency department: which test is best? *Pediatrics*. 1998;101(6):E1.
7. Gorelick MH, Shaw KN. Screening tests for urinary tract infection in children: A meta-analysis. *Pediatrics*. 1999;104(5):e54.
8. Bachur R, Harper MB. Reliability of the urinalysis for predicting urinary tract infections in young febrile children. *Arch Pediatr Adolesc Med*. 2001;155:60–5.
9. Dayan PS, Hanson E, Bennett JE, et al. Test characteristics of the urine Gram stain in infants <or= 60 days of age with fever. *Pediatr Emerg Care*. 2002;18:12–4.
10. Glissmeyer EW, Korgenski EK, Wilkes J, et al. Dipstick screening for urinary tract infection in febrile infants. *Pediatrics*. 2014;133:e1121-7.
11. Schroeder AR, Chang PW, Shen MW, et al. Diagnostic accuracy of the urinalysis for urinary tract infection in infants <3 months of age. *Pediatrics*. 2015; 135:965–71.
12. Chaudhari PP, Monuteaux MC, Bachur RG. Urine Concentration and Pyuria for Identifying UTI in Infants. *Pediatrics*. 2016;138:e20162370.
13. McGillivray D, Mok E, Mulrooney E, et al. A head-to-head comparison: “clean-void” bag versus catheter urinalysis in the diagnosis of urinary tract infection in young children. *J Pediatr*. 2005;147(4):451-456.
14. Selekman RE, Sanford MT, Ko LN, et al. Does perception of catheterization limit its use in pediatric UTI? *J Pediatr Urol*. 2017;13(1):48.e1-48.e6.
15. Ouellet-Pelletier J, Guimont C, Gauthier M, et al. Adverse Events Following Diagnostic Urethral Catheterization in the Pediatric Emergency Department. *CJEM*. 2016;18(6):437-442.
16. Paragas N, Kulkarni R, Werth M, et al. α-Intercalated cells defend the urinary system from bacterial infection. *J Clinical Invest.* 2014; 124(7):2963-76.
17. Parravicini E, Nemerofsky SL, Michelson KA, et al. Urinary neutrophil gelatinase-associated lipocalin is promising biomarker for late onset culture-positive sepsis in very low birth weight infants. *Pediatr Res.* 2010;67:636–40.
18. Mishra J, Ma Q, Prada A, et al. Identification of neutrophil gelatinase-associated lipocalin as a novel early urinary biomarker for ischemic renal injury. *J Am Soc Nephrol*. 2003;14:2534-43.
19. Zappitelli M, Washburn KK, Arikan AA, et al. Urine neutrophil gelatinase-associated lipocalin is an early marker of acute kidney injury in critically ill children: a prospective cohort study. *Crit Care.* 2007;11:R84.
20. Wheeler DS, Devarajan P, Ma Q, et al. Serum neutrophil gelatinase-associated lipocalin (NGAL) as a marker of acute kidney injury in critically ill children with septic shock. *Crit Care Med.* 2008;36:1297–303.
21. Bagshaw SM, Bennett M, Haase M, et al. Plasma and urine neutrophil gelatinase-associated lipocalin in septic versus non-septic acute kidney injury in critical illness. *Intensive Care Med.* 2010;36(3):452–61.
22. Paragas N, Qiu A, Hollmen M, et al. NGAL-Siderocalin in kidney disease. *Biochim Biophys Acta.* 2012;1823:1451–8.
23. Vanmassenhove J, Glorieux G, Lameire N, et al. Influence of severity of illness on neutrophil gelatinase-associated lipocalin performance as a marker of acute kidney
24. Steigedal M, Marstad A, Haug M, et al. Lipocalin 2 imparts selective pressure on bacterial growth in the bladder and is elevated in women with urinary tract infection. *J Immunol.* 2014; 193:6081–9.
25. Czaja C. A., Stamm W. E., Stapleton A. E., et al. Prospective cohort study of microbial and inflammatory events immediately preceding Escherichia coli recurrent urinary tract infection in women. *J Infect Dis.* 2009;528–36.
26. Yilmaz A, Sevketoglu E, Gedikbasi A, et al. Early prediction of urinary tract infection with urinary neutrophil gelatinase associated lipocalin. *Pediatr Nephrol.* 2009;24:2387–92.
27. Yim HE, Yim H, Bae ES, et al. Predictive value of urinary and serum biomarkers in young children with febrile urinary tract infections. *Pediatr Nephrol.* 2014; 29:2181-9. 23. Lee H-E, Kim DK, Kang HK, Park K. The diagnosis of febrile urinary tract infection in children may be facilitated by urinary biomarkers. *Pediatr Nephrol*. 2015;30(1):123-130.
28. Lee H-E, Kim DK, Kang HK, Park K. The diagnosis of febrile urinary tract infection in children may be facilitated by urinary biomarkers. *Pediatr Nephrol*. 2015;30(1):123-130.

29. Ichino M, Kuroyanagi Y, Kusaka M, et al. Increased urinary neutrophil gelatinase associated lipocalin levels in a rat model of upper urinary tract infection. *J Urol.* 2009;181:2326.
30. Lubell TR, Barasch JM, Xu K, et al. Urinary Neutrophil Gelatinase-Associated Lipocalin for the Diagnosis of Urinary Tract Infections. *Pediatrics*. 2017;140(6):e20171090.
31. Valdimarsson S, Jodal U, Barregård L, et al. Urine neutrophil gelatinase-associated lipocalin and other biomarkers in infants with urinary tract infection and in febrile controls. *Pediatr Nephrol*. July 2017.
32. Paragas N, Qiu A, Zhang Q, et al. The Ngal reporter mouse detects the response of the kidney to injury in real time. *Nat Med.* 2011;17:216–22.
33. Forster CS, Jackson E, Ma Q, et al. Predictive ability of NGAL in identifying urinary tract infection in children with neurogenic bladders. *Pediatr Nephrol*. March 2018.
34. Lubell TR, Schnadower D, Freedman SB, et al. Comparison of Febrile Infants with Enterococcal and Gram-negative Urinary Tract Infections. Pediatr Infect Dis J. 2016; 35(9):943-948.
35. Parikh CR, Butrymowicz I, Yu A, et al. Urine stability studies for novel biomarkers of acute kidney injury. *Am J Kidney Dis*. 2014;63(4):567-572.
36. Schuh MP, Nehus E, Ma Q, et al. Long-term Stability of Urinary Biomarkers of Acute Kidney Injury in Children. *Am J Kidney Dis*. 2016;67(1):56-61.
37. Singer E, Elger A, Elitok S, et al. Urinary neutrophil gelatinase-associated lipocalin distinguishes pre-renal from intrinsic renal failure and predicts outcomes. *Kidney Int*. 2011;80(4):405-414.
38. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. *Biometrics*. 1988;44(3):837-845.