UIJ UroToday International Journal®

www.urotodayinternationaljournal.com
Volume 4 - August 2011

Evaluation of Biomarkers to Differentiate Upper From Lower Urinary Tract Infections in Children

Ahmed J Al-Sayyad,[1] Salah M EL-Morshedy,[2] Ehab A abd Al Hamid,[2] Nehad A Karam,[3] A Basset A Imam,[2] Rehab A Karam[4]

[1]Urology Department, King Abdulaziz University Hospital, Jeddah, Saudi Arabia; [2]Pediatrics Department, King Abdulaziz University Hospital, Jeddah, Saudi Arabia, [3]Pediatrics Department, Faculty of Medicine, Zagazig University, Egypt; [4]Biochemistry Department, Faculty of Medicine, Zagazig University, Egypt

Submitted April 10, 2011 - Accepted for Publication May 25, 2011

ABSTRACT

INTRODUCTION: Distinguishing upper from lower urinary tract infections (UTI) has important clinical implications in children, especially in those younger than 2 years of age. The objective of this study was to test differences between upper and lower UTIs by using serum and urine biomarkers.

METHODS: Participants were 83 patients with UTI based on suggestive clinical symptoms and at least 1 positive urine culture. All had renal scintigraphy. Children with known concomitant diseases, any type of renal disorder, or a previous diagnosis of vesicoureteral reflux were excluded. Before the initiation of antibiotic treatment, blood was sampled for white blood cell (WBC) count, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and procalcitonin (PCT). Urinary interleukin-6 (uIL-6) and macrophage migration inhibitory factor (MIF) were also measured. Data were analyzed using the Mann-Whitney or *t* test. Sensitivity and specificity were calculated for some variables in isolation and in combination.

RESULTS: There were 61 girls and 22 boys with mean (SD) age of 8.7 (3.4) months and 7.8 (4.5) months, respectively; 49 patients had acute pyelonephritis (APN) and 34 had lower UTI. The mean WBC counts were significantly higher in the group with APN than in the group with lower UTI ($P < .01$), as were CRP and ESR levels ($P < .001$). Significantly higher serum PCT, urinary IL-6, and MIF levels were detected in patients with APN when compared with patients with lower UTI (all with $P < .001$). For the prediction of APN, sensitivity and specificity levels were 95.9% and 88.2% for CRP, 87.8% and 91.2% for PCT, 71.4% and 94.1% for uIL-6, and 93.9% and 97.1% for urinary MIF. The sensitivity and specificity for CRP combined with other biomarkers were 93.9% and 91.2% (PCT with CRP), 95.9% and 91.2% (uMIF with CRP), and 85.7 % and 94.1% (uIL-6 with CRP), respectively.

CONCLUSION: Some biomarkers, used solely or in combination, help to differentiate between upper and lower UTI and may make more aggressive and invasive testing unnecessary in the future.

Abbreviations and Acronyms

APN, acute pyelonephritis
CRP, C-reactive protein
DMSA, dimercaptosuccinic acid
ELISA, enzymelinked immunosorbent assay
ESR, erythrocyte sedimentation rate
IL-6, interleukin-6
MIF, migration inhibitory factor
PCT, procalcitonin
RPI, renal parenchymal involvement
UTI, urinary tract infection
WBC, white blood cell

KEYWORDS: Urinary tract infection; Acute pyelonephritis; Biomarkers

CORRESPONDENCE: Ahmed Al-Sayyad MD, FRCSC, Assistant Professor and Consultant of Pediatric Urology, King Abdulaziz University, PO Box 1817 Jeddah 21441, Saudi Arabia (aboassil@yahoo.com).

CITATION: *UroToday Int J*. 2011 Aug;4(4):art49. doi:10.3834/uij.1944-5784.2011.08.05

UroToday International Journal®

original study

Evaluation of Biomarkers to Differentiate Upper From Lower Urinary Tract Infections in Children

INTRODUCTION

Urinary tract infection (UTI) is one of the most common bacterial infections in children and may involve the upper tract (acute pyelonephritis) and/or the lower urinary tract (cystitis/urethritis). The differentiation between upper and lower UTI has important clinical implications in children, especially in those younger than 2 years of age. The dimercaptosuccinic acid (DMSA) scan is currently the gold standard for diagnosing acute pyelonephritis (APN). A meta-analysis of animal studies using DMSA to diagnose APN showed an overall sensitivity of 86% and a specificity of 91% [1]. However, DMSA is not readily available in all centers. It also exposes the patients to radiation and may not differentiate between old scarring and acute parenchymal involvement.

Currently, diagnosis of APN is based on clinical manifestations and determination of acute-phase reactants in blood [2-4]. Defense against mucosal infections relies on chemokines that recruit inflammatory cells to the mucosa [5]. Proinflammatory cytokines, particularly interleukin-6 (IL-6) determined in urine (uIL-6) or serum (sIL-6), have been shown to be useful as parametric biological indicators of renal involvement in UTI. Macrophages infiltrate the renal parenchyma during all types of renal injury, including renal inflammation, and their numbers correlate with the intensity of inflammation [6,7].

Macrophage migration inhibitory factor (MIF) is a proinflammatory cytokine that is a potent activator of macrophages and T cells [8-10]. This factor may play a crucial role in initiating or maintaining several inflammatory conditions [11]. Procalcitonin (PCT) and proinflammatory cytokines (TNF-a, IL-6, and IL-1b) increase as acute-phase reactants during infections. PCT, a polypeptide identical to a prohormone of calcitonin, was initially described as a potential marker of bacterial diseases [12].

The objective of the present study was to differentiate between APN and lower UTI using different serum and urine biomarkers.

METHODS

This prospective study was approved by the King Abdulaziz University Hospital Ethics Committee. All patients provided informed consent. The study was conducted from March 2009 through December 2010.

Participants

A total of 83 patients with UTI were included in our study. Diagnosis of UTI was based on suggestive clinical symptoms and at least 1 positive urine culture, as defined previously [13]. Exclusion criteria included children with: (1) known concomitant diseases (eg, allergies, rheumatological illnesses, neoplasms), (2) any type of renal disorder of immunological, malformative or inflammatory origin, or (3) a previous diagnosis of vesicoureteral reflux.

Lower UTI was defined as UTI without involvement of the renal parenchyma and with normal renal scintigraphy [14]. APN was defined as UTI with involvement of the renal parenchyma that was suspected through clinical symptoms (eg, high temperature, lumbar pain) and confirmed by the presence of specific abnormalities on renal DMSA scan [14,15]. APN was diagnosed using DMSA when: (1) the renal contour was normal or had a protuberance, (2) the renal size was normal or enlarged without loss of volume, and (3) there was a single or multiple hypocaptation and/or the percentage of renal captation was less than 45%.

Procedures

Before the initiation of antibiotic treatment, blood was sampled for white blood cell (WBC) count, neutrophil count, C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR). Clotted blood (4 mL) was centrifuged; the serum was separated and frozen at -70°C prior to analysis in order to measure PCT and cytokine levels. PCT was measured in serum using immunoluminometric methods with kits (BRAHMS PCT LUMItest; Berlin, Germany).

Urine MIF concentration was quantified using enzymelinked immunosorbent assay (ELISA) according to the manufacturer's instructions (R & D Systems; Minneapolis, MN, USA). UIL-6 (pg/mL) levels were determined by ELISA using commercial kits and following the manufacturer's instructions (Bender MedSystems Diagnostics/eBioscience; San Diego, CA, USA; Boehringer-Ingelheim GmbH, Ingelheim am Rein, Germany).

Data Analysis

Cutoff levels for a positive response were defined as: CRP > 3.5 mg/dL; PCT > 0.85 ng/mL; uIL6 > 15 pg/mL; and uMIF > 450 pg/dL. Collected data were presented as mean, standard deviation (SD), range, and percentages. Data were analyzed using the Mann-Whitney or *t* test using SPSS for Windows 11 (IBM Corp; Somers, NY). Statistical significance was set at $P < .05$. Sensitivity and specificity were calculated for some variables in isolation and in combination.

RESULTS

The study included 83 patients; there were 61 girls and 22 boys with mean (SD) age of 8.7 (3.4) months and 7.8 (4.5) months, respectively. In this study, 49 (59%) patients had APN and 34

doi:10.3834/uij.1944-5784.2011.08.05
ISSN 1944-5792 (print), ISSN 1944-5784 (online)

Table 1. Biochemical Data From Patients With Acute Pyelonephritis or Lower Urinary Tract Infection; Probability of Significant Differences (N = 83).
doi: 10.3834/uij.1944-5784.2011.08.05t1

Variable	Group				*P*
	Acute Pyelonephristis		Lower Urinary Tract Infection		
	Mean	SD	Mean	SD	
White blood cell count, K/uL	16.5	5.6	8.7	3.4	< .01[a]
Erythrocyte sedimentation rate, mm/H	51.1	25.3	15.0	6.5	< .001[a]
C-reactive protein, mg/dL	9.5	3.5	3.1	2.1	< .001[a]
Procalcitonin, ng/mL	3.5	1.9	0.2	0.85	< .001[b]
Urine interleukin-6, pg/mL	15.2	10.5	2.5	3.4	< .001[b]
Urine migration inhibitory factor, pg/dL	3155.45	344.43	265.43	85.6	< .001[a]

[a]*t* test
[b]Mann-Whitney test

(41%) had lower UTI. There was no significant difference in the mean age of patients with lower UTI and APN. We found that 79% of UTIs were caused by *Escherichia coli* and the remainder by other microorganisms (*Klebsiella, Proteus, Enterobacter*, and *Staphylococcus aureus*).

Table 1 contains the biochemical results from patients with APN and lower UTI. The mean WBC counts were significantly higher in the group with APN than in the group with lower UTI (P < .01), as were CRP and ESR levels (P < .001). Significantly higher serum PCT levels were detected in patients with APN when compared with patients with lower UTI (P < .001). Urinary IL-6 and MIF levels were also significantly higher in the group with APN (P < .001).

Table 2 contains the sensitivity and specificity of PCT, IL-6, uIL-6, and uMIF for patients with APN. For the prediction of APN on admission, CRP had a sensitivity of 95.9% and a specificity of 88.2%. The sensitivity and specificity of PCT were 87.8% and 91.2%, respectively. Urinary IL-6 had a sensitivity of 71.4% and a specificity of 94.1%. The sensitivity and specificity of uMIF were 93.9% and 97.1%, respectively.

Different biomarker combinations with CRP were tried to study the possible improvement of sensitivity and specificity in distinguishing between APN and lower UTI. Results are contained in Table 3. The sensitivity and specificity for combinations were 93.9% and 91.2% (PCT with CRP), 95.9% and 91.2% (uMIF with CRP), and 85.7% and 94.1% (uIL-6 with CRP), respectively.

DISCUSSION

The early diagnosis of APN in children is a challenge in the absence of specific symptomatology, particularly during infancy. The early appreciation of abnormal renal tissue using DMSA, especially in infants and children younger than 5 years, has already been described [16]. Differentiation of APN from lower UTI in infants and children is necessary because renal

Table 2. Sensitivity and Specificity of C-reactive Protein, Procalcitonin, Urinary Interleukin-6, and Urinary Migration Inhibitory Factor for Acute Pyelonephritis.
doi: 10.3834/uij.1944-5784.2011.08.05t2

Cut-Off Values	Sensitivity %	Specificity %	Positive Predictive Value	Negative Predictive Value
C-reactive protein > 3.5 mg/dL	95.9	88.2	92.2	93.8
Procalcitonin > 0.85 ng/mL	87.8	91.2	91.5	83.8
Urine interleukin-6 > 15 pg/mL	71.4	94.1	94.6	61.6
Urine migration inhibitory factor > 450 pg/dL	93.9	97.1	97.9	91.7

Table 3. Sensitivity and Specificity of Biomarkers Combined With C-reactive Protein to Distinguish Between Acute Pyelonephritis and Lower Urinary Tract Infection.
doi: 10.3834/uij.1944-5784.2011.08.05t3

Combination	Sensitivity %	Specificity %	Positive Predictive Value	Negative Predictive Value
C-reactive protein + procalcitonin	93.9	91.2	93.9	91.2
C-reactive protein + urine migration inhibitory factor	95.9	97.1	97.9	94.3
C-reactive protein + urine interleukin-6	85.7	94.1	95.5	82.1

parenchymal involvement can induce scarring that may lead to arterial hypertension and chronic renal failure. To achieve this differentiation, we measured proinflammatory cytokines and PCT levels as well as urinary IL-6 and urinary MIF.

As in all infectious diseases, WBC, ESR, and CRP are expected to increase as acute-phase reactants during UTI. In our study, there was a highly significant difference between upper and lower urinary tract infection regarding those parameters ($P < .001$).

Our data indicate that serum PCT concentration has high sensitivity and specificity for the diagnosis of APN and allows accurate differentiation from children with lower UTI. Our results are in agreement with a study done by Kotoula et al [17] in which 27 children were diagnosed as having renal parenchymal involvement (RPI) based on positive renal scintigraphy. The authors found that PCT was more sensitive and specific than ESR or CRP for the diagnosis of upper versus lower UTI. Moreover, PCT had the highest sensitivity, specificity, and positive and negative predictive values (89%, 97%, 96%, and 91%, respectively). Furthermore, the results of 2 other previous studies [18,19] showed that PCT (determined by either an immunoluminometric quantitative or a rapid semiquantitative test) was elevated in children with APN and often normal in children with lower UTI, with a sensitivity of 70.3% to 74% and a specificity of 82.6% to 85%. These results are consistent with our findings. The data from these previous studies suggest that PCT is a reliable marker for detecting RPI in children with APN [18,19]. Conversely, 2 studies [20,21] showed that PCT is neither sensitive nor specific for the early diagnosis of upper UTI. This variation in results may be due to age and sex differences in the populations studied and/or to the techniques used to diagnose UTI.

IL-6 is a proinflammatory cytokine; serum levels of IL-6 increase in response to bacterial infection [7,22]. Serum and urinary levels of this protein increase during UTI, and its measurement has been shown to be useful in differentiating between APN and lower UTI [7,23]. Measurement of urinary IL-6 is less aggressive than determination of serum levels and therefore more acceptable to patients and health professionals. Our study confirms the value of using urinary IL-6 to differentiate between upper and lower UTIs in children. The present study also provides new information on the value of uIL-6 as a diagnostic indicator of UTI. Our results show that urinary concentrations of IL-6 >15 pg/mL indicate APN in children with suspected clinical manifestations with a specificity of 94.1% and sensitivity of 71.4%. Therefore, the use of more aggressive diagnostic tests appears to be unnecessary. This finding is in agreement with Rodríguez et al [24], who stated that specificity for uIL-6 (according to the cutoff point established by a receiver operating characteristic curve) was 94.1% (95% CI: 91.1–97.1). Moreover,uIL-6 was undetectable when the children became asymptomatic and finished their antibiotic treatment course, independent of the UTI site.

Gurgoze et al [25] reported results that are comparable to those of our study, using serum IL-6 as an indicator for distinguishing APN from lower UTI. The sensitivity of uIL-6 in the diagnosis of APN, using the calculated cutoff point, was lower than that reported for sCRP and sIL-6. This means that determination of uIL-6 will yield a higher percentage of false negatives among patients with APN. However, specificity for a value of uIL-6 > 15 pg/mL is much greater than that for sIL-6 and sCRP [25]. Thus, 87 out of 100 UTI patients with uIL-6 >15 pg/mL have APN and the rest are false positives.

MIF is a proinflammatory cytokine that is a potent activator of macrophages and T cells [9–11]. This factor may play a crucial role in initiating or maintaining several inflammatory conditions [12]. In experimental models, treatment with antibodies to MIF was able to reverse or prevent colitis and gastric ulcer formation [26,27].

Our study also confirmed the utility of determining urinary MIF. Levels > 450 pg/dL achieved a specificity of 97.9% and

sensitivity of 93.9% in differentiating between upper and lower UTI in children during the acute phase of the disease. This is in agreement with Otukesh et al [28], who studied urinary MIF in 25 children with acute pyelonephritis, 8 children with acute cystitis, and 40 controls without UTI. He used a cut-off point of uMIF/Cr > 4.89 pg/µmol to differentiate upper from lower UTI and found a sensitivity and specificity of 92% and 100%, respectively. A major drawback of this study was the small number of patients with lower UTI. In another study, Sevketoglu et al [29] investigated a larger group of children with acute cystitis (n = 56), acute pyelonephritis (n = 4), and healthy controls (n = 30). They found that 295 pg/mL for uMIF can be used to predict UTI in children. However, they were unable to determine a cut-off point to differentiate between upper and lower UTI because of the limited number of patients with APN.

Combining some biomarkers with CRP appears to increase sensitivity and specificity. When we combined CRP with PCT, uIL6, and uMIF, the sensitivity and specificity of CRP with uMIF was 95.9% and 97.1%, respectively. These results were greater than any other parameter combined with CRP.

CONCLUSION

Some biomarkers, used solely or in combination, help to differentiate between upper and lower UTI and may make more aggressive testing unnecessary in the future. Larger caliber prospective studies will be needed to confirm this finding.

Conflict of Interest: none declared.

REFFERENCES

1. Craig JC, Wheeler DM, Irwig L, Howman-Giles RB. How accurate is dimercaptosuccinic acid scintigraphy for the diagnosis of acute pyelonephritis? A meta-analysis of experimental studies. *J Nucl Med*. 2000;41(6):986-993.

2. Robles Garcia B, Rodríguez Fernandez LM, Suárez Rodriguez MA, Marugan de Miguelsanz JM, Fernández M, Fuentes MC. Comparison of the usefulness of fever and some laboratory tests for the diagnosis of acute pyelonephritis in children [article in Spanish]. *Rev Esp Pediatr.* 2005;61:358-363.

3. Ataei N, Madani A, Habibi R, Khorasani M. Evaluation of acute pyelonephritis with DMSA scans in children presenting after the age of 5 years. *Pediatr Nephrol*. 2005;20(10):1439-1444.

4. Shah G, Upadhyay J. Controversies in the diagnosis and management of urinary tract infections in children. *Paediatr Drugs*. 2005;7(6):339-346.

5. Godaly G, Otto G, Burdick MD, Strieter RM, Svanborg C. Fimbrial lectins influence the chemokine repertoire in the urinary tract mucosa. *Kidney Int*. 2007;71(8):778-786.

6. Gürgöze MK, Akarsu S, Yilmaz E, et al. Proinflammatory cytokines and procalcitonin in children with acute pyelonephritis. *Pediatr Nephrol*. 2005;20(10):1445-1448.

7. Rodríguez LM, Marugán JM, Suárez MA, Torres MC. Cytokines in pediatric nephrourologic pathology [article in Spanish]. *Arch Latinoam Nefrol Ped*. 2003;3(2):73-81.

8. Gervaix A, Galetto-Lacour A, Gueron T, et al. Usefulness of procalcitonin and C-reactive protein rapid tests for the management of children with urinary tract infection. *Pediatr Infect Dis J*. 2001;20(5):507-511.

9. Morand EF, Bucala R, Leech M. Macrophage migration inhibitory factor: an emerging therapeutic target in rheumatoid arthritis. *Arthritis Rheum*. 2003;48(2):291-299.

10. Metz CN, Bucala R. Role of macrophage migration inhibitory factor in the regulation of the immune response. *Adv Immunol*. 1997;66:197-223.

11. Metz CN, Bucala R. MIF. In: Oppenheim JJ, Feldman M, eds. *Cytokine Reference: A Compendium of Cytokines and Other Mediators of Host Defense*. Vol 1. San Diego, CA: Academic Press; 2001:703-716.

12. Karzai W, Oberhoffer M, Meier-Hellmann A, Reinhart K. Procalcitonin--a new indicator of the systemic response to severe infections. *Infection*. 1997;25(6):329-334.

13. Espinosa L. Infección urinaria. In: Garcia Nieto V, Santos F, Rodríguez Iturbe B, eds. *Nefrología Pediátrica.* 2nd ed. Madrid: Aula Médica; 2006:507-520.

14. Harbert JC, Eckelman WC, Neumann RD. *Nuclear Medicine, Diagnosis and Therapy*. 1st ed. New York, NY:Thieme Medical; 1996:713-743.

15. Patel K, Charron M, Hoberman A, Brown ML, Rogers KD. Intra- and interobserver variability in interpretation of DMSA scans using a set of standardized criteria. *Pediatr Radiol*. 1993;23(7):506-509.

16. Lin KY, Chiu NT, Chen MJ, et al. Acute pyelonephritis and sequelae of renal scar in pediatric first febrile urinary tract infection. *Pediatr Nephrol.* 2003;18(4):362-365.

17. Kotoula A, Gardikis S, Tsalkidis A, et al. Procalcitonin for the early prediction of renal parenchymal involvement in children with UTI: preliminary results. *Int Urol Nephrol*. 2009;41(2):393-399.

18. Benador N, Siegrist CA, Gendrel D, et al. Procalcitonin is a marker of severity of renal lesions in pyelonephritis. *Pediatrics*. 1998;102(6):1422-1425.

19. Gervaix A, Galetto-Lacour A, Gueron T, et al. Usefulness of procalcitonin and C-reactive protein rapid tests for the management of children with urinary tract infection. *Pediatr Infect Dis J*. 2001;20(5):507-511.

20. Tuerlinckx D, Vander Borght T, Glupczynski Y, et al. Is procalcitonin a good marker of renal lesion in febrile urinary tract infection? *Eur J Pediatr*. 2005;164(10):651-652.

21. Güven AG, Kazdal HZ, Koyun M, et al. Accurate diagnosis of acute pyelonephritis: How helpful is procalcitonin? *Nucl Med Commun*. 2006;27(9):715-721.

22. Marugán Miguelsanz JM, Suárez Rodriguez MA, Rodríguez Fernandez LM, García Ruiz De Morales JM. Normal levels of interleukin 6 and 8 in serum and urine of healthy asymptomatic children [article in Spanish]. *Bol Pediatr*. 2005;45:177-184.

23. Sheu JN, Chen MC, Lue KH, et al. Serum and urine levels of interleukin-6 and interleukin-8 in children with acute pyelonephritis. *Cytokine*. 2006;36(5-6):276-282.

24. Rodríguez LM, Robles B, Marugán JM, Suárez A, Santos F. Urinary interleukin-6 is useful in distinguishing between upper and lower urinary tract infections. *Pediatr Nephrol.* 2008;23(3):429-433.

25. Gürgöze MK, Akarsu S, Yilmaz E, et al. Proinflammatory cytokines and procalcitonin in children with acute pyelonephritis. *Pediatr Nephrol*. 2005;20(10):1445-1448.

26. Huang XR, Chun Hui CW, Chen YX, et al. Macrophage migration inhibitory factor is an important mediator in the pathogenesis of gastric inflammation in rats. *Gastroenterology.* 2001;121(3):619-630.

27. Ohkawara T, Nishihira J, Takeda H, et al. Amelioration of dextran sulfate sodium-induced colitis by anti-macrophage mig*ration inhibitory factor antibody in mice.* Gastroenterology. 2002;123(1):256-270.

28. Otukesh H, Fereshtehnejad SM, Hoseini R, et al. Urine macrophage migration inhibitory factor (MIF) in children with urinary tract infection: a possible predictor of acute pyelonephritis. *Pediatr Nephrol*. 2009;24(1):105-111.

29. Sevketoglu E, Yilmaz A, Gedikbasi A, et al. Urinary macrophage migration inhibitory factor in children with urinary tract infection. *Pediatr Nephrol*. 2010;25(2):299-304.