# Validity of Neutrophil Gelatinase Associated Lipocaline as a Biomarker for Diagnosis of Children with Acute Pyelonephritis

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**Purpose:** Novel biomarkers have been investigated for various renal disorders, including urinary tract infection (UTI). The aim of this study was to assess whether urine neutrophil gelatinase associated lipocaline (NGAL), could represent a reliable biomarker for diagnosis and treatment of children with acute pyelonephritis (APN).

**Materials and Methods:** A total of 37 children (32 females, 5 males) with APN were included in this prospective study. Urine NGAL was measured before and 5-7 days after antibiotic treatment in the UTI group, using ELISA kit and compared with 26 (8 females, 18 males) control group children admitted for other bacterial infections.

**Results:** Mean age of the UTI group was 39 ± 28 months, compared to 43.6 ± 31.5 months for the control group with no statistically significant difference. Median urine NGAL level was significantly higher in patients with APN than the other subjects [0.48 (interquartile range (IQR): 0.15-0.72) vs. 0.065 (0.01-0.24), $P = .001$], and decreased significantly after antibiotic treatment ($P = .002$).
Using a cutoff of 0.20 ng/mL, sensitivity and specificity of urine NGAL were 76% and 77% for prediction of APN, respectively. The area under the ROC curve (AUC) for urine NGAL was 0.75 (CI= 0.61-0.88), suggesting urine NGAL as a relatively good predictive biomarker of APN.

**Conclusion:** Urine NGAL is a good biomarker for diagnosis and treatment monitoring of APN in children.

**Keywords:** NGAL; acute pyelonephritis; children; diagnosis; treatment

## INTRODUCTION

Urinary tract infection (UTI) is one of the most common serious bacterial infections in early life. Early diagnosis and treatment of acute pyelonephritis (APN) is important to prevent its long-term complications, including renal scars, hypertension, and chronic renal failure[(1)] . However, more severe forms of UTI may cause dehydration and subsequent prerenal azotemia[(2)]. Although urine culture has been considered the gold standard test for diagnosis of UTI, positive culture requires 2–3 days for identification of the responsible organism with false positive and negative results[(3)]. In addition, sensitivity and specificity of urinary tract symptoms, pyuria, nitrite test, leukocyte esterase (LE), WBC, ESR, and CRP are low and do not accurately localize upper and lower UTI[(4-7)]. DMSA scan have been considered as the gold standard test for diagnosis of APN in recent years. However, based on new guidelines for the evaluation of APN, DMSA scan has been recommended for screening of renal damage 3-6 months following the acute phase of infection[(8)].
Therefore, it is necessary to develop a sensitive, rapid and noninvasive test with therapeutic implications for early diagnosis of APN, especially in suspicious patients[(9)]. Neutrophil gelatinase associated lipocalin (NGAL) is a recently investigated biomarker for diagnosis of acute kidney injury. It is a component of the innate immune system[(6)], which reduces bacterial growth in the early phase of inflammation[(5)], and has been identified in different human tissues, including renal proximal tubules and neutrophil granules[(9,10,11)]. Increased urine NGAL concentration facilitated rapid diagnosis of APN in the absence of acute kidney injury and chronic kidney disease in the recent investigations [(6,9,10)]. The aim of this study was to determine the value of urine NGAL for early prediction of children with APN, compared to the other bacterial infections.

## MATERIALS AND METHODS

This is a hospital based case-control study, conducted over a 1-year period between 2014 and 2015. It was approved by the institutional ethics committee and informed consent was obtained from parents. Inclusion criteria consisted of 37 consecutive children less than 12 years admitted for APN with no other infectious

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**Table 1.** Demographic and clinical characteristics of the study groups

| | Case | Control | *P*-value |
|---|---|---|---|
| Subject (%) | 37(59) | 26(41) | |
| NGAL ± SD | .47 ± .34 | .22 ± .34 | 0.001[a] |
| Age ± SD | 39.0 ± 28 | 43.6 ± 31.5 | 0.55[a] |
| Fever ± SD | 38.3 ± .81 | 38.6 ± 0.36 | 0.029[a] |
| WBC ± SD | 13827 ± 5170 | 12538 ± 4623 | 0.3[b] |
| ESR ± SD | 43 ± 22 | 37 ± 19 | 0.27[b] |
| Male/Female (%) | 5/32(13.5/86.5) | 18/8(69/31) | <0.001[c] |
| Antibiotic administration (%) | 23 (62) | 19 (73) | 0.366[c] |

[a] Mann-Whitney *U* test, [b] independent *T* test, [c] chi-square test

or inflammatory conditions (case group). A total of 26 age matched children admitted for other bacterial infections such as meningitis, pneumonia, septicemia or septic arthritis with no history of urological complaints were considered as control group. APN was defined as positive urine culture (any growth in suprapubic aspiration, > 105 CFU/mL of a single pathogen in urine bag collection, or > 104 CFU/mL by uretheral catheterization) associated with fever >38.5°C, leukocyte count more than the normal value according to age, positive CRP, increased ESR, and pyuria (urine WBC > 5/hpf). Patients with known urologic or anorectal malformations, decreased renal function, recent antibiotic treatment, recent history of urological intervention, single kidney, associated infections and inflammatory disorders, neurologic disorders, and immunodeficiency were excluded from the study.

Based on new guidelines, DMSA scan was performed in patients with abnormal ultrasound, atypical UTI, or confirmation of suspicious APN, which showed pyelonephritic changes as focal or diffuse areas of decreased cortical uptake, with the preservation of renal contour in 60% of patients[8]. Urine sample was obtained in both groups before antibiotic treatment and 5-7 days after treatment in the UTI group, and frozen at –80°C. Urine NGAL was measured using a commercially available ELISA kit (BioPorto Diagnostics, Gentofte Denmark), according to the manufacturer's instructions, and expressed as ng/mL.

### *Statistical analysis*

Sample size was calculated based on the previous study[9] with sensitivity and specificity of 90 and 92%, respectively. It was determined by 0.8 estimated power and 95% confidence level. Statistical analysis was performed using SPSS software version 22 (Chicago, IL, USA). Normality of continuous variables was assessed by Kolmogorov-Smirnov test. Normally distributed continuous variables were assessed by independent sample t-test, whereas Mann-Whitney U test was used for group comparison of non- normal continuous variables. Chi-square test was used to evaluate qualitative binary data. Predictive factors of APN were assessed by univariate and multivariate analysis on variables with *P*-value ≥ 0.2. Crude and adjusted OR were obtained by stepwise backward logistic regression. Removal probability < 0.1 was considered for stepwise analysis. Receiver operating curve (ROC) analysis was used to determine optimal cot-off point of sensitivity and specificity. Comparison of ROC curve has also been performed by STATA SE software version 11.

**Table 2.** Univariate and multivariable analysis of characteristics associated with the presence of acute pyelonephritis

| | Univariable analysis | | Multivariable analysis | |
|---|---|---|---|---|
| | OR(CI) | *P* | Adjusted OR(CI) | *P* |
| NGAL | 8.9(1.67-47.2) | 0.01 | 5.2(0.74-35) | 0.09 |
| Age | 0.99(.976- 1.01) | 0.54 | - | - |
| Fever | 0.49(0.21-1.1) | 0.085 | 0.36(0.13-1.006) | 0.05 |
| WBC | 1 | 0.3 | - | - |
| ESR | 1.01(0.98-1.04) | 0.27 | - | - |
| [a]Sex | 0.069(0.02-0.24) | <0.001 | 0.079(0.02-0.31) | < 0.001 |
| Antibiotic administration | 1.6(0.55-4.9) | 0.37 | - | - |

[a]female is reference

**Table 3.** Sensitivity, specificity and area under the curve (AUC) for optimal cut-off values of NGAL, fever and sex compared with full model for diagnosis of acute pyelonephritis

| | Sensitivity | Specificity | AUC | Cut off | *P*-value |
|---|---|---|---|---|---|
| Full Model | 97.3 | 69.23 | 84(0.72-0.95) | - | reference |
| NGAL | 75.68 | 76.92 | 75(0.61-0.88) | > = 0.2 | 0.12 |
| Fever | 54.05 | 88.46 | 66(0.52-0.79) | | 0.08 |
| Sex | 86.5 | 69.2 | - | - | - |

## RESULTS

Totally, 37 patients in the APN group and 26 in the control group were enrolled in this study. Mean age of cases was 39 ± 28 months compared to 43.6 ± 31.5 months in the control group, with no significant difference ($P$ = 0.55). Female gender was higher in APN compared to the control group (86.5% vs. 31%). Median urine NGAL level was significantly higher in patient with APN than the control group [(0.48 (interquartile range (IQR): 0.15-0.72) vs. 0.065 (0.01-0.24), $P$ = .001]. **Table 1** demonstrates demographic and laboratory variables in the cases and controls. Mean initial urine NGAL was 0.46 ± 0.35 and decreased significantly to 0.25 ± 0.27 after antibiotic treatment ($P$ = .002). Urine NGAL and gender were associated in univariate analysis. However, fever and gender were significant independent variables in multivariate analysis (**Table 2**). The best cutoff level of urine NGAL for predicting APN was 0.2 ng/mL. Using these cutoff points, the sensitivity and specificity of urine NGAL were 74% and 67%, respectively (**Table 3**). The area under the ROC curve (AUC) for urine NGAL was 0.75 (CI= 0.61-0.88), suggesting urine NGAL as a relatively good predictive biomarker of APN (**Figure 1**). There was no significant difference in the AUC between reference final model, fever ($P$ = .08) and urine NGAL ($P$ = .12) (**Table 3**).

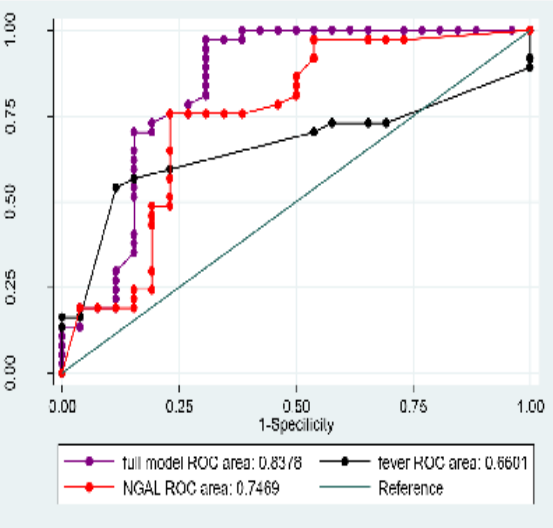


**Figure 1.** Diagnostic characteristics of NGAL, fever in comparison with the full model (NGAL, Fever, and Sex)

## DISCUSSION

This study was performed to evaluate the potential value of urine NGAL for prediction of children with APN. We confirmed significant increase of urine NGAL during the acute phase of APN, which wasndowngraded with the appropriate treatment. The value of serum and urine NGAL for diagnosis and therapeutic monitoring of febrile UTI have been recently reported. In a similar study, Arambasic et al. showed higher level of urine NGAL in children with APN compared to acute cystitis and other febrile infections. It was considered a useful biomarker for diagnosis of APN in children[12]. In an experimental model of APN, urine NGAL increased in the early phase of acute inflammation following cortical injection of Ecoli, suggesting urine NGAL as a new biomarker of APN[13]. Urine NGAL was a specific test for evaluation of APN in Ghasemi et al. study, compatible with DMSA scan grading and CRP level. They recommended measurement of other common biomarkers such as ESR, leukocyte count, and CRP combined with urine NGAL for the prediction of renal parenchymal involvement[14]. In Lee et al. study, urine NGAL was in accordance to the acute photon defects of DMSA renal scan, obviating imaging studies in children with low urine NGAL level[9]. DMSA scan was performed in half of our patients, and nonsignificant correlations may not reflect the true incidence. However, urine NGAL increased in both upper and lower UTI, with no differentiation between these two groups in the other reports[3]. Similar to our study, urine NGAL significantly decreased 3–4 days after antibiotic treatment[7,9] and have been considered as predictive biomarker for therapeutic monitoring of APN[6]. Persistently elevated urine NGAL may be associated with treatment unresponsiveness in children with acute febrile UTIs[7]. We found urine NGAL as a relatively sensitive (74%), specific (67%) and accurate test (AUC = 0.75) for diagnosis of children with APN, which was similar to Yim et al. study with 75% sensitivity and 73.7% specificity[6]. However, it was a more valid biomarker for diagnosis of UTI with 97% sensitivity and 76% specificity in Yilmaz et al. report[5]. Urine NGAL had lower or equal sensitivity and specificity for diagnosis of APN, compared to leukocyte esterase; 99% versus 70%, bacteriuria; 81% versus 83%[8], urine nitrite; 53% versus 98%, and pyuria; 73% versus 81% in our study, respectively. Increased serum NGAL concentration have been also detected as an early biomarker of APN in patients with acute bacterial infections[6]. Serum NGAL was a rapid

and sensitive test for prediction of APN with a significant correlation with DMSA renal cortical defect in the acute phase of febrile UTI in Seo et al. study[7], and excluded radiologic evaluation in lower level. Other studies showed a correlation between plasma NGAL level with duration of fever, WBC, CRP, and creatinine level[15]. In conclusion, urine NGAL was a relatively sensitive and accurate biomarker for differentiation of APN from other infectious disorders. However, compared to the other parameters, we recommended screening of APN with simple inexpensive traditional urinalysis, and urine NGAL is suggested to identify highly suspicious patients with false negative results, differentiate contamination from urinary tract infection, in addition to serve as a therapeutic biomarker. This study provides a small piece of evidence that urine NGAL excretion could be considered in further and larger populations to confirm the potential application of this biomarker.

## CONCLUSIONS:

Urine NGAL is a good biomarker for diagnosis and treatment monitoring of APN in children.

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## CONFLICT OF INTEREST

The authors have declared that no conflict of interest exists.

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