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Neutrophil Gelatinase-Associated Lipocalin: A Biomarker for Early Diagnosis of Urinary Tract Infections in Infants

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Abstract

Early diagnosis of urinary tract infection (UTI) is challenging in infants due to unspecific symptoms, difficulty in urine collection and possible contamination. The aim of this study was to assesses the usefulness of serum and urine neutrophil gelatinase-associated lipocalin (sNGAL and uNGAL, respectively) in the diagnosis of febrile and non-febrile UTI in infants. This prospective observational study enrolled 66 infants with the first episode of UTI and 18 healthy controls. At the time of enrollment, sNGAL, uNGAL, urinalysis, urine culture, white blood cell count (WBC), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), procalcitonin (PCT), and serum creatinine (sCr) were assessed. We found that, on average, both sNGAL and uNGAL levels were significantly higher in febrile UTI, compared to non-febrile UTI and controls. In turn, the mean sNGAL level, but not uNGAL, was significantly higher in the non-febrile UTI group compared to controls. sNGAL positively correlated with WBC, CRP, ESR and PCT, and uNGAL with CRP and leukocyturia. The receiver operating curves (ROC) demonstrate that the optimum cut-off of 76.2 ng/ml for sNGAL (sensitivity 92.9%, specificity 94.4%, and the area under the curve (AUC) of 0.98) and of 42.2 ng/ml for uNGAL (sensitivity 73.8%, specificity 72.2%, and AUC of 0.76) for diagnosing febrile UTI and 39.0 ng/ml for sNGAL (sensitivity 83.3%, specificity 55.6%, and AUC of 0.70) for diagnosing non-febrile UTI. In conclusion, serum NGAL is an excellent marker for the early diagnosis of febrile UTI, with sensitivity and specificity higher than those of urine NGAL. Diagnostic sensitivity of serum NGAL is smaller in non-febrile infants suffering from UTI, and urine NGAL is not useful for this purpose at all.

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1 Introduction

Urinary tract infection (UTI) is one of the most common bacterial infection in children (Stein et al. 2015). Urine culture is the gold standard for diagnosing UTI, but culture results require at least 2 d for a complete identification of bacteria (Ylmaz et al. 2009). Therefore, results of urinalysis, such as the presence of leukocyturia, and nitrite and leukocyte esterase, are used to diagnose UTI in clinical practice, but low sensitivity and specificity of this analysis is an issue (Kim et al. 2014). Difficulty in urine collection or improperly collected specimen may lead to false positive results of urine culture and urinalysis in infants (Hatipoglu et al. 2011). Additionally, leukocyturia in infants may be a sign of a wide range of inflammatory changes, including perineum, vaginitis, diarrhoea, synechia, or phimosis. Sterile leukocyturia may also occur in noninfectious conditions such as urolithiasis, congenital abnormalities of kidney and urinary tract (CAKUT), recent history of urological intervention, dehydratation, and high fever of external causes (Szmigielska and Krzemień 2017; AAP 2011).

Early diagnosis of UTI in infants is especially important, because a delay in diagnosis and treatment can result in severe renal parenchymal involvement and can increase the risk of chronic kidney damage, including renal failure (Ghasemi et al. 2016; Sim et al. 2015). Therefore, novel blood and urine biomarkers are needed for an early diagnosis of UTI in children (Kim et al. 2014; Yim et al. 2014).

Neutrophil gelatinase-associated lipocalin (NGAL) is an important component of the innate antimicrobial immune system (Ylmaz et al. 2009). The main bacteriostatic effect of NGAL on gram-negative bacteria is binding and sequestration of iron-loaded bacterial siderophores, which prevents bacterial iron uptake and growth. NGAL also modulates neutrophil-related processes such as maturation, adhesion, phagocytosis, or bacterial killing and it acts as chemoattractant for neutrophils. In healthy individuals, NGAL is expressed at very low concentrations in neutrophils and in epithelial cells of several tissues including kidney, liver, lungs, and colon (Nasioudis and Witkin 2015). In acute kidney injury, NGAL is massively released into the blood and urine from nephron epithelium (Singer et al. 2013). NGAL also is an acute phase reactant and may be released from activated neutrophils and other immune cells (Kim et al. 2014). Usefulness of plasma and urine NGAL (pNGAL and uNGAL) measurements for diagnosing chronic kidney damage and different bacterial infection has been reported in various studies (Filho et al. 2017; Singer et al. 2013). Recent data show that serum NGAL (sNGAL), alongside pNGAL and uNGAL assay, can be used for prediction of UTI (Yim et al. 2014; Ylmaz et al. 2009), acute pyelonephritis (Kim et al. 2017; Nickavar et al. 2016), or renal scarring (Rafiei et al. 2015). Clinical studies assessing the usefulness of NGAL in UTI diagnosis in children have been performed in a small number of patients, are single-center studies, and the results are contentious (Ghasemi et al. 2016; Lee et al. 2015; Kim et al. 2014). Therefore, the aim of the present study was to assess the usefulness of sNGAL and uNGAL in the early diagnosis of febrile and non-febrile UTI in infants.

2 Methods

This prospective observational study was performed in the Department of Pediatric and Nephrology of Warsaw Medical University in Warsaw, Poland of over a period of 3 years. The study was approved by a local Bioethics Committee for Human Research. All parents gave their informed consent. Eighty four children (42 males, 42 females, mean age 5.8 ±3.4 months, range 1–12 months) were enrolled in the study: 66 infants with a first episode of UTI and 18 healthy infants as a control group. Children with other infections, symptoms of chronic kidney damage, chronic kidney damage, liver dysfunction, evidence of obstructive nephropathy, and moderate- or severe-grade of vesicoureteral reflux were excluded from the study. Based on the clinical manifestation and laboratory tests, children were divided into three groups: febrile UTI, non-febrile UTI, and healthy controls. Urinalysis, urine culture, white blood cell count (WBC), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), procalcitonin (PCT), and serum creatinine (sCr) were performed on admission. Urine samples were collected *via* a catheter or from a midstream urine. Leukocyturia was defined as >10 WB *per* high power field in the urine sediment. Significant bacteriuria was defined as growth of a single pathogen $\geq 10^5$/colony-forming units *per* ml urine. Normal values of the indices evaluated in the local laboratory were as follows: WBC $\leq$ 18.000/mm^3, CRP $\leq$ 1.0 mg/dl, ESR $\leq$ 15 mm/h, PCT $\leq$ 0.05 ng/ml, and sCr $\leq$ 0.4 mg/dl. The UTI diagnosis was based on significant bacteriuria and leukocyturia. Febrile UTI was defined as the presence of fever (≥38.0 °C). Serum and urine samples collected for the measurement of NGAL were obtained at the time of admission. They were immediately centrifuged and stored at –80 °C until further analysis.

Urine and serum NGAL content was determined using enzyme-linked immunoadsorbent assay (ELISA) kit (Human Lipocalin-2/NGAL; Cat no: RD 191102200R; BioVendor Laboratory Medicine Inc., Brno, Czech Republic). The detection range for NGAL was 0.3–10 ng/ml. Urine NGAL to urine creatinine ratio (uNGAL/uCr) was calculated to normalize samples and was expressed as ng/mg. Renal ultrasound was performed after admission. Voiding cystourethrography (VCUG) was performed 2–3 weeks after treatment of UTI in children with any abnormal finding in renal ultrasound.

2.1 Statistical Evaluation

The Liliefors and Shapiro-Wilk tests were used to determine whether the data were normally distributed. Continuous data were presented as means ±SD or median and interquartile ranges (IQ). Categorical data were presented as numbers and percentiles. One-way ANOVA or a non-parametric Kruskal-Wallis test were used to compare continuous variables between the three groups. A *post hoc* test was used to compare subgroups. A chi-squared test was used to compare categorical variables. Correlations between variables were evaluated by linear regression analysis or Sperman's rank correlation. The receiver operating curves (ROC) were made and areas under the curves (AUC) were calculated, including 95% confidence intervals (CI), to determine sensitivity and specificity, and the optimal cut-off values of sNGAL, uNGAL, and uNGAL/uCr ratio to predict UTI. A p-value <0.05 defined statistically significant differences. Statistical evaluation was performed using a commercial Statistica package v11.0 for Windows (StatSoft; Tulssa, OK).

3 Results

Febrile UTI was diagnosed in 42 (64%) and non-febrile UTI in 24 (36%) children. The control group consisted of 18 children without UTI. *Escherichia coli* was isolated in 60 (91%) children with UTI, and other bacteria in 6 (9%) children (*Klebsiella sp.* in two, *Enterococcus sp.* in two, and *Proteus sp.* in two children). Indications

Table 1 Demographic, laboratory, and imaging findings in children with febrile and non-febrile urinary tract infection (UTI), and in controls

	Group			p		
Variable	Febrile (1) (n = 42)	Non-febrile (2) (n = 24)	Controls (3) (n = 18)	1 *vs.* 2	1 *vs.* 3	2 *vs.* 3
Age (month)	7.1 ± 3.4	3.9 ± 2.6	5.2 ± 3.0	<0.001	<0.05	ns
Male, *n* (%)	25 (59.5)	9 (37.5)	8 (44.4)	ns	ns	ns
sNGAL (ng/ml)	159.6 ± 63.9	60.6 ± 19.9	46.3 ± 22.2	<0.0001	<0.0001	<0.05
uNGAL (ng/ml)	67.5 ± 34.8	48.0 ± 41.1	38.0 ± 32.3	<0.05	<0.01	ns
uNGAL/uCr	172. 0	195.2	178.3	ns	ns	ns
IQ	116.5 – 267.9	85.8 – 371.5	44.1 – 338.0			
↑ WBC, *n* (%)	25 (59)	5 (19)	0	<0.001	<0.0001	<0.05
↑ CRP, *n* (%)	42 (100)	14 (58)	0	<0.001	<0.0001	<0.001
↑ ESR, *n* (%)	36 (100)	14 (64)	0	<0.001	<0.0001	<0.0001
↑ PCT, *n* (%)	42 (100)	14 (58)	0	<0.001	<0.0001	<0.001
WBC (mm^3)	20.4 ± 6.2	14.5 ± 4.3	11.2 ± 2.5	<0.001	<0.0001	<0.01
CRP (mg/dl)	8.8	1.5	0.5	<0.0001	<0.0001	<0.0001
IQ	5.7 – 17.3	0.6 – 2.9	0.5 – 0.5			
ESR (mm/h)	72.0 ± 31.4	27.3 ± 18.1	9.1 ± 4.3	<0.0001	<0.0001	<0.05
PCT (ng/ml)	0.96	0.08	0.05	<0.0001	<0.0001	<0.01
IQ	0.34–2.33	0.05–0.09	0.05–0.05			
Leukocyturia (uWBC/HPF)	100.0	55.0	1.0	ns	<0.0001	<0.0001
IQ	30.0 – 100.0	27.5 – 100.0	0.0 – 3.0			
CAKUT, *n* (%)	5 (11)	5 (21)	4 (22)	ns	ns	ns

Data are presented as means ± SD, median with interguartile range (IQ), or number (%); *sNGAL* serum neutrophil gelatinase-associated lipocalin, *uNGAL* urine neutrophil gelatinase-associated lipocalin, *uNGAL/uCr* urine NGAL to urine creatinine ratio, *WBC* white blood cell count, *CRP* serumC-reactive protein, *ESR* erythrocyte sedimentation rate (performed in 76 children), *PCT* procalcitonin, ↑ number of patients with elevated values, *uWBC/HPF* urine WBC/high power field, *CAKUT* congenital anomaly of kidney and urinary tract

for urinalysis and urine culture in the control group included the following: failure to thrive (three), prolonged jaundice (two), and abnormalities in the urinary tract unraveled in ultrasonography (four). Routine urine tests were performed in 9 children. In all children in the control group, urine culture was negative and urinalysis was normal. sCr was normal in all children evaluated in the study.

A comparison of demographic, laboratory and imaging findings in children with febrile and non-febrile UTI, and in healthy controls are presented in Table 1. The children with febrile UTI were significantly older than those with both non-febrile UTI and healthy controls. There was no significant difference in age in children with non-febrile UTI compared to controls. Gender distribution did not differ between the groups. The mean sNGAL and uNGAL levels were significantly higher in the febrile UTI group than those in both non-febrile UTI and controls. The mean sNGAL, but not uNGAL, was significantly higher in the non-febrile UTI than that in controls. There were no significant differences in the median uNGAL/uCr ratio among the groups of children.

Elevated values of WBC, CRP, ESR i PCT were present significantly more often in the febrile UTI than those in the non-febrile UTI and control groups, and in the non-febrile UTI than in the control group. WBC, CRP, ESR and PCT were significantly higher in the febrile UTI than those in the non-febrile UTI and control groups, and in the non-febrile UTI than in the control group. No significant differences in the urine WBC count were documented between children with febrile and non-febrile UTI. Based on renal ultrasound and voiding

Table 2 Correlations between sNGAL, uNGAL, uNGAL/uCr ratio and age, systemic inflammatory markers, leukocyturia

	sNGAL		uNGAL		uNGAL/uCr	
Variable	*r*	p	*r*	p	*r*	p
Age	0.33	<0.01	–	–	−0.26	<0.05
uNGAL	–	–	–	–	0.53	<0.001
WBC	0.70	<0.001	–	–	–	–
CRP	0.67	<0.001	0.30	<0.01	–	–
ESR	0.59	<0.001	–	–	–	–
PCT	0.53	<0.001	–	–	–	–
Leukocyturia	–	–	0.36	<0.01	–	–

sNGAL serum neutrophil gelatinase-associated lipocalin, *uNGAL* urine neutrophil gelatinase-associated lipocalin, *uNGAL/uCr* urine NGAL to urine creatinine ratio, *WBC* white blood cell count, *CRP* C-reactive protein, *ESR* erythrocyte sedimentation rate, *PCT* procalcitonin

Table 3 Area under the curve (AUC), optimal cut-off value, sensitivity and specificity for sNGAL, uNGAL, and uNGAL/uCr ratio for the diagnosis of febrile and non-febrile UTI

Variable	AUC	Z-test	p	Cut-off	Sensitivity	Specificity
Febrile UTI						
sNGAL	0.98 (95% CI 0.96–1.00)	39.5	<0.00001	76.2	92.9%	94.4%
uNGAL	0.76 (95% CI 0.61–0.89)	3.43	<0.001	42.2	73.8%	72.2%
uNGAL/uCr	0.55 (95% CI 0.38–0.73)	0.58	ns	–	–	–
Non-febrile UTI						
sNGAL	0.70 (95% CI 0.54–0.87)	2.38	<0.05	39.0	83.3%	55.6%
uNGAL	0.56 (95% CI 0.39–0.74)	0.71	ns	–	–	–
uNGAL/uCr	0.55 (95% CI 0.37–0.73)	0.55	ns	–	–	–

sNGAL serum neutrophil gelatinase-associated lipocalin, *uNGAL* urine neutrophil gelatinase-associated lipocalin, *uNGAL/uCr* urine NGAL to urine creatinine ratio, *UTI* urinary tract infection

cystouretrography, CAKUT was diagnosed in 14 (16.7%) children, including five with febrile UTI (four children with vesicoureteral reflux Grade 2 and one child with duplex kidney), five with non-febrile UTI (four children with mild hydronephrosis and one child with duplex kidney), and in four children mild hydronephrosis from the control group. There were no significant differences in frequency of CAKUT among the groups of children.

Correlations between sNGAL, uNGAL, uNGAL/uCr, one side and age, systemic inflammatory markers, and leukocyturia on the other side, are presented in Table 2. Positive correlations were present between uNGAL and uNGAL/uCr. sNGAL positively correlated with age, WBC, CRP, ESR, and PCT. uNGAL positively correlated only with CRP and leukocyturia. sNGAL correlation with age was lower than that with inflammatory markers. uNGAL/uCr was inversely correlated with age, and uCr was positively correlated with age. Gender had no appreciable influence on sNGAL or uNGAL levels (data not shown).

Area under the curve calculated with the ROC method, optimal cut-off value, sensitivity and specificity for sNGAL, uNGAL, and uNGAL/uCr for diagnosing of febrile and non-febrile UTI are shown in Table 3. ROC analysis demonstrated that sNGAL had the greatest AUC value of 0.98 for diagnosing febrile UTI, followed by uNGAL of 0.76 for diagnosing febrile UTI, and sNGAL of 0.70 for diagnosing non-febrile UTI.

4 Discussion

In infants, early diagnosis of UTI is often challenging, because of nonspecific symptoms of infection, difficulty in urine collection and a high risk of contamination (Lee et al. 2013; Tullus 2011). Inadequately collected specimens can lead to misdiagnosis and to unnecessary antibiotic treatment in children with false positive changes (Krzemień et al. 2014; Hatipoglu et al. 2011). Thus, there is a need for sensitive and specific laboratory tests for accurate diagnose of UTI in infants and young children. Ichino et al. (2009) have demonstrated in an experimental study that uNGAL increases in the early stage of UTI, suggesting that uNGAL might be used for predicting UTI. Since that time, usefulness of blood and urine NGAL assessment in UTI have been investigated in animal and human studies (Forster et al. 2017; Kim et al. 2017; Urbschat et al. 2014).

The main source of blood NGAL in UTI are activated neutrophiles in the infected kidney and activated peripheral neutrophiles in case of neutrophilia (Lee et al. 2015; Kim et al. 2014). In patients with severe form of acute pyelonephritis and concomitant chronic kidney damage, NGAL is released from injured nephron epithelia into blood and urine (Singer et al. 2013). Urine NGAL derives mainly from activated neutrophiles present in urine and in the infected kidney, less from injured nephron epithelia (Decavele et al. 2011).

In the present study we assessed sNGAL and uNGAL in a cohort of infants with UTI and found significantly higher sNGAL and uNGAL levels in infants with febrile UTI compared to non-febrile UTI and healthy controls, and a significantly higher sNGAL level in infants with non-febrile UTI compared to the controls. Ylmaz et al. (2009) have demonstrated that uNGAL increases in children diagnosed with UTI. Those authors have evaluated 60 children with symptomatic UTI and reported that uNGAL and uNGAL/uCr are significantly higher in the UTI group than in controls. They have also determined the optimum cut-off value of 20 ng/ml for uNGAL (sensitivity 97%, specificity 76%, and AUC 0.98) and of 30 for uNGAL/uCr (sensitivity 98%, specificity 76%, and AUC 0.99) for diagnosing UTI and concluded that uNGAL and uNGAL/uCr are sensitive markers for early UTI diagnose in the absence of chronic kidney damage or chronic kidney damage. In a similar study, Yim et al. (2014) have investigated 73 children with febrile UTI and also found that uNGAL and uNGAL/uCr are significantly higher in the UTI group than in controls. Using the optimum cut-off value of 23.95 ng/ml for uNGAL (sensitivity 82.4%, specificity 83.6%, and AUC 0.89) and of 276.5 for uNGAL/uCr (sensitivity 76%, specificity 90%, and AUC 0.90), they noted smaller sensitivity, but greater specificity of both markers for an early UTI diagnosis than those in the of Ylmaz et al. (2009). It is worth noting that the optimum cut-off values of uNGAL normalized to uCr in both studies are different. These differences are due most likely to different age of children in both studies. Children studied by Yim et al. (2014) were younger than those studied by Ylmaz et al. (2009). Additionally, it is known that concentration of uNGAL is high and concentration of uCr is low during infancy.

Another study has demonstrated a significantly higher level of uNGAL/Cr in 33 children with febrile UTI than in controls (Lee et al. 2015). With the best cut-off value of 108 ng/mg, uNGAL/uCr shows a good diagnostic profile for UTI (sensitivity 97%, specificity 80.6%, and AUC 0.91). A study by Urbschat et al. (2014), performed in 59 adults with clinical symptoms of upper and lower UTI, has revealed a significantly higher uNGAL level in patients with UTI than in controls. Arambašić et al. (2016) have noted that uNGAL can be useful for the differentiation of cystitis from febrile states other than UTI. Hatipoglu et al. (2011) have assessed a complex of matrix metalloproteinase-9 (MMP-9) and NGAL in 71 children with lower UTI and in 37 with an asymptomatic contamination. Those authors report that the uMMP-9/NGAL/uCr level

is significantly higher in the UTI group, but it is unelevated in asymptomatic children with contaminated urine and in healthy controls. Using the optimum cut-off value 0.08 ng/mg for uMMP-9/NGAL/Cr (sensitivity 98.6%, specificity 97.3%, and AUC 0.99) they found higher sensitivity and specificity values of this marker for the diagnosis of UTI, than those reported by Lee et al. (2015), Yim et al. (2014), and Ylmaz et al. (2009) for uNGAL and uNGAL/uCr.

According to the reports above outlined, uNGAL, uNGAL/Cr, and uMMP-9/uNGAL/uCr demonstrate high sensitivity, specificity, and AUC value for the diagnosis of UTI. The increases in those markers were associated with UTI severity and they dropped significantly following antibiotic treatment. The markers were useful for diagnosing and managing febrile UTI in children, and also for the differentiation between contamination and infection. In Ylmaz et al. (2009) and Hatipoglu et al.'s (2011) opinions, when using the optimum cut-off value for the markers, treatment can be started even before the results of urine culture become available. In patients with persistently elevated uNGAL/uCr, there is a risk of inappropriate treatment of febrile UTI. Therefore, Yim et al. (2014) have recommended dimercaptosuccinic acid (DMSA) scans for these patients to exclude renal scars. There is a consensus that uNGAL and uNGAL/uCr levels cannot replace the urinalysis and urine culture in UTI diagnosis, but measurements of these markers can improve the management algorithms.

In the present study we found lower sensitivity and specificity of uNGAL (73.8% and 72.2%, respectively) for diagnosing febrile UTI than those in the studies above outlined. We also found that uNGAL was not useful for diagnosing non-febrile UTI. In contrast, results of some other studies have demonstrated high sensitivity of uNGAL normalized for uCr for predicting UTI. Normalization of urine biomarkers for uCr is a common practice, although it is a debatable approach (Bennett et al. 2015; McWilliam et al. 2014). In chronic kidney damage, the absolute values of biomarkers can be misleading due to a difference in urinary flow rate. Likewise, normalized values of biomarkers can be misleading due to variations in uCr excretion (Waikar et al. 2010). Forster et al. (2017) have suggested that in patients with UTI, uNGAL normalization for uCr is an imperfect standardization due to distinctly different biologies of uNGAL and uCr. In patients with UTI, uNGAL is released from injured tubular cells or neutrophiles present in urine, whereas uCr is depended on the glomerular filtration rate. In present study, uNGAL/uCr was inversely associated with age. Normalization of uNGAL for uCr did not improve the prognostic value of this biomarker in UTI diagnosis. This finding is in line with those from other studies (Rafiei et al. 2015; Kim et al. 2014; Yim et al. 2014).

In children, the measurement of urine biomarkers is preferred over blood tests as it is an easier and less stressful procedure. Therefore, only a few investigators assessed sNGAL or pNGAL in children with UTI. Most of them evaluated the usefulness of NGAL measurements to predict acute pyelonephritis in patients with febrile UTI (Kim et al. 2017; Sim et al. 2015). Seo et al. (2014) have evaluated 47 infants with febrile UTI and found a significantly higher pNGAL level than that in controls. The authors conclude that the use of rapid pNGAL assay at the time of admission can help predict the clinical course of febrile UTI in infants and determine treatment strategies. Urbschat et al. (2014) have reported a higher sNGAL level in adults with UTI accompanied by fever than in those without fever. A study of Petrovic et al. (2013) performed in 50 children with febrile UTI, demonstrates a significantly higher sNGAL level at admission than that at hospital discharge.

Conflicting data have been reported by Kim et al. (2014), who investigated 107 children with UTI and 337 children without UTI. In this large-scale pediatric study, sNGAL and uNGAL were elevated in the UTI compared to non-UTI group, but the difference between the two groups was statistically insignificant. When the comparison

included the presence or absence of pyuria, the mean sNGAL and uNGAL content was significantly greater in the pyuria-posifive than that in pyuria-negative group. However, the optimum cut-off value 82.5 ng/ml for sNGAL (sensitivity 70%, specificity 60%, and AUC 0.67) and 10.3 ng/ml for uNGAL (sensitivity and specificity 70%, and AUC 0.78) demonstrated rather low sensitivity and specificity for diagnosing a pyuria-positive UTI.

Studies of Urbschat et al. (2014) and Kim et al. (2014) have demonstrated that uNGAL is more useful than sNGAL for the UTI diagnosis. In contrast, in the present study we found higher sensitivity and specificity of sNGAL than uNGAL for the febrile UTI diagnosis. Since a normal result of urinalysis does not enable the exclusion of UTI (Ghasemi et al. 2016; Salleeh et al. 2010; Etoubleau et al. 2009; Readorn et al. 2009), we conclude that sNGAL may be useful for the UTI diagnosis in febrile infants having no changes in urinalysis and for the differentiation of UTI from febrile states other than UTI. In most studies, urine and blood NGAL content was investigated in children with febrile UTI. We evaluated also infants with non-febrile UTI, because the UTI diagnosis and differentiation between contamination and infection is still a challenge at that especially vulnerable age. sNGAL was less sensitive for the non-febrile than febrile UTI, and uNGAL was unuseful for the non-febrile UTI diagnosis. In the present study, we also found a significantly higher level of systemic inflammatory markers in children with the febrile than non-febrile UTI, and in children with the non-febrile UTI compared to controls. We demonstrate that sNGAL associated with the level of WBC, CRP, ESR, and PCT, and uNGAL associated with the level of CRP and leukocyturia.

Although numerous studies have demonstrated an association between pNGAL, sNGAL, and uNGAL, on the one side, and WBC and CRP, on the other side (Ghasemi et al. 2016; Lee et al. 2015; Sim et al. 2015; Urbschat et al. 2014; Yim et al. 2014), other studies have found no such relationships (Seo et al. 2014; Hatipoglu et al. 2011; Ylmaz et al. 2009). Various studies have also demonstrated the association of sNGAL and uNGAL markers with leukocyturia and leukocyte esterase (Lee et al. 2015; Kim et al. 2014; Cullen et al. 2012; Decavele et al. 2011; Hatipoglu et al. 2011), but again some other studies reported no such relationships (Ylmaz et al. 2009). Seo et al. (2014) have speculated that NGAL content increases at the time of chronic kidney damage from bacterial infection, and decreases after clinical improvement. We opine that the association of sNGAL and uNGAL with fever and inflammatory markers, and a decrease in sNGAL and uNGAL after onset of treatment, support the notion that NGAL enhancement is induced by inflammatory process rather than by the kidney epithelial injury.

The limitations of our study include its design as a single-center study, a small number of patients, and a difference in children's age between the groups. However, it is challenging to have closely age-matched groups in observational, comparative studies. In this study, the age difference amounted to 2–3 months, which was rather small, despite being statistically significant. Therefore, it seems rather unlikely that a high content of serum and urine NGAL, we noted in children with febrile UTI, would be associated with the age-difference rather than with the severity of UTI.

In conclusion, this prospective, observational study demonstrates that serum NGAL is an excellent marker for the diagnosis of febrile UTI, having high sensitivity and specificity. Among the febrile infants, serum NGAL is a more sensitive marker for an early UTI diagnosis than urine NGAL. Among the non-febrile infants, serum NGAL is less sensitive for the UTI diagnosis and urine NGAL is not good to this end at all. In our opinion, serum NGAL may be a very useful marker for the UTI diagnosis in febrile infants, having normal results of urinalysis. Serum NGAL seems particularly useful for the differentiation between UTI and febrile states other than UTI-related and between infection and contamination in infants with a suspicion of UTI.

Conflicts of Interest The authors declare no conflicts of interest in relation to this article.

References

AAP (2011) American Academy of Pediatrics. Subcommittee on Urinary Tract Infection, Steering Committee on Quality Improvement and Management. Urinary Tract Infection: Clinical practice guideline for the diagnosis and management of the initial UTI in febrile infants and children 2 to 24 months. Pediatrics 128:595–610

Arambašić J, Mandić S, Debeliak Ž, Mandić D, Horvat V, Šerić V (2016) Differentiation of acute pyelonephritis from other febrile states in children using urinary neutrophil gelatinase-associated lipocalin (uNGAL). Clin Chem Lab Med 54(10):55–61

Bennett MR, Nehus E, Haffner C, Ma Q, Devarajan P (2015) Pediatric reference ranges for acute kidney injury biomarkers. Pediatr Nephrol 30:677–685

Cullen MR, Murray PT, Fitzgibbon MC (2012) Estabilishment of a reference interval for urinary neutrophil gelainase-associated lipocalin. Ann Clin Biochem 49:190–193

Decavele AS, Dhondt L, De Buyzere ML, Delanghe JR (2011) Increased urinary neutrophil gelainase associated lipocalin in urinary tract infections and leukocyturia. Clin Chem Lab Med 49(6):999–1003

Etoubleau C, Reveret M, Brouet D, Badier L, Brosset P, Fourcade L, Bahans C, Garnier F, Blanc P, Guigonis V (2009) Moving from bag to catheter for urine collection in non-toilet-trained children suspected of having urinary tract infection: a paired comparison of urine cultures. J Pediatr 154:803–806

Filho LT, Grande AJ, Colonetti T, Colonetti T, Della ÉSP, da Rosa MI (2017) Accuracy of neutropfil gelatinase-associated lipocalin for acute kidney injury diagnosis in children: systematic review and meta-analysis. Pediatr Nephrol. https://doi.org/10.1007/s00467-017-3704-6

Forster CS, Johnson K, Patel V, Wax R, Rodig N, Barasch J, Bachur R, Lee R (2017) Urinary NGAL deficiency in recurrent urinary tract infections. Pediatr Nephrol 32:1077–1080

Ghasemi K, Esteghamati M, Borzoo S, Parvaneh E, Borzoo S (2016) Predictive accuracy of urinary neutrophil gelatinase associated lipocalin (NGAL) for renal parenchymal involvement in children with acute pyelonephritis. Electron Physician 8:1911–1917

Hatipoglu S, Sevketoglu E, Gedikbasi A, Ylmaz A, Kiyak A, Mulazimoglu M, Aydogan G, Ozpacaci T (2011) Urinary MMP-9/NGAL complex in children with acute cystitis. Pediatr Nephrol 26:1263–1268

Ichino M, Kuroyanagi Y, Kusaka M, Mori T, Ishikawa K, Shiroki R, Kurahashi H, Hoshinaga K (2009) Increased urinary neutrophil gelatinase associated lipocalin levels in a rat model of upper urinary tract infection. J Urol 181(5):2326–2331

Kim BH, Yu N, Kim HR, Yun KW, Lim IS, Kim TH, Lee M-K (2014) Evaluation of the optimal neutrophil gelatinase-associated lipocalin value as a screening biomarker for urinary tract infections in children. Ann Lab Med 34:354–359

Kim BK, Yim HE, Yoo KH (2017) Plasma neutrophil gelatinase-associated lipocalin: a marker of acute pyelonephritis in children. Pediatr Nephrol 32:477–484

Krzemień G, Szmigielska A, Artemiuk I, Roszkowska-Blaim M (2014) False positive urine cultures in children under two years of age – own research. Dev Period Med 18(2):172–177

Lee H-E, Lee SH, Baek M, Choi H, Park K (2013) Urinary measurement of neutrophil gelatinase associated lipocalin and kidney injury molecule-1 helps diagnose acute pyelonephritis in a preclinical model. J Biomarkers 2013:6

Lee H-E, Kim DK, Kang HK, Park K (2015) The diagnosis of febrile urinary tract infection in children may be facilitated by urinary biomarkers. Pediatr Nephrol 30:123–130

McWilliam SJ, Antoine DJ, Sabbisetti V, Pearce RE, Jorgensen AL, Lin Y, Leeder JS, Bonventre JV, Smyth RL, Primohamed M (2014) Reference intervals for urinary renal injury biomarkers KIM-1 and NGAL in healthy children. Biomark Med 8:1189–1197

Nasioudis D, Witkin SS (2015) Neutrophil gelatinase-associated lipocalin and innate immune responses to bacterial infections. Med Microbiol Immunol 204:471–479

Nickavar A, Safaeian B, Valavi E, Moradpour F (2016) Validity of neutrophil gelatinase associated lipocaline as a biomarker for diagnosis of children with acute pyelonephritis. Pediatr Urol 13(5):2860–2863

Petrovic S, Bogavac-Stanojevic N, Peco-Antic A, Ivanisevic I, Kotur-Stevuljevic J, Paripovic D, Sopic M, Jelic-Ivanovic Z (2013) Clinical application neutrophil-associated lipocalin and kidney injury molecule-1 as indicators of inflammation persistence and acute kidney injury in children with urinary tract infection. Biomed Res Int 2013:947157

Rafiei A, Mohammadjafari H, Bazi S, Mirabi AM (2015) Urinary neutrophil gelatinase-associated lipocalin (NGAL) might be an independent marker for anticipating scar formation in children with acute pyelonephritis. J Renal Inj Prev 4:39–44

Readorn JM, Carstairs KL, Rudinsky SL, Simon LV, Riffenburgh RH, Tanen AD (2009) Urinalysis is not reliable to detect a urinary tract infection in febrile infants presenting to the ED. Am J Emerg Med 27:930–932

Salleeh H, McGillivray D, Martin M, Patel H (2010) Duration of fever affects the likelihood of positive bag urinalysis or catheter culture in young children. J Pediatr 156:629–633

Seo WH, Nam SW, Lee EH, Je B-K, Yim HE, Choi BM (2014) A rapid plasma neutrophil gelatinase-associted lipocalin assay for diagnosis of acute pyelonephritis in infants with acute febrile urinary tract infections: a preliminary study. Eur J Pediatr 173:229–232

Sim JH, Yim HE, Choi BM, Lee JH, Yoo KH (2015) Plasma neutrophil gelatinase-associated lipocalin predicts acute pyelonephritis in children with urinary tract infection. Pediatr Res 78:48–55

Singer E, Marko L, Paragas N, Barasch N, Dragun D, Müller DN, Budde K, Schmidt-Ott KM (2013) Neutrophil gelatinase-associated lipocalin: pathophysiology and clinical applications. Acta Physiol 207 (4):663–672

Stein R, Dogan HS, Hoebeke P, Kočvara R, Nijman RJM, Radmayr C, Tekgül S, European Association of Urology, European Society for Pediatric Urology (2015) Urinary tract infection in children: EAU/ESPU guidelines. Eur Urol 67:546–558

Szmigielska A, Krzemień G (2017) Sterile leukocyturia – difficult diagnostic problem in children. Dev Period Med 21(2):108–112

Tullus K (2011) Difficulties in diagnosing urinary tract infections in small children. Pediatr Nephrol 26:1923–1926

Urbschat A, Obermüller PP, Reissig M, Hadji P, Hofmann R, Geiger H, Gauer S (2014) Upper and lower urinary tract infections can be detected early but not be descriminated by urinary NGAL in adults. Int Urol Nephrol 46:2243–2249

Waikar SS, Sabbisetti VS, Bonventre JV (2010) Normalization of urinary biomarkers to creatinine during changes in glomerular filtration rate. Kidney Int 78 (5):486–496

Yim HE, Yim H, Bae ES, Woo SU, Yoo KH (2014) Predictive value of urinary and serum biomarkers in young children with febrile urinary tract infections. Pediatr Nephrol 29:2181–2189

Ylmaz A, Sevketoglu E, Gedikbasi A, Karyagar S, Kiyak A, Mulazimoglu M, Aydogan G, Ozpacaci T, Hatipoglu S (2009) Early prediction of urinary tract infection with urinary neutrophil gelatinase associated lipocalin. Pediatr Nephrol 24:2387–2392