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ORIGINAL ARTICLE

Urine soluble TLR4 levels may contribute to predict urinary tract infection in children: the UTILISE Study

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Abstract

Background One of the most common bacterial infections in childhood is urinary tract infection (UTI). Toll-like receptors (TLRs) contribute to immune response against UTI recognizing specific pathogenic agents. Our aim was to determine whether soluble TLR4 (sTLR4), soluble TLR5 (sTLR5) and interleukin 8 (IL-8) can be used as biomarkers to diagnose UTI. We also aimed to reveal the relationship between urine Heat Shock Protein 70 (uHSP70) and those biomarkers investigated in this study.

Methods A total of 802 children from 37 centers participated in the study. The participants ($n=282$) who did not meet the inclusion criteria were excluded from the study. The remaining 520 children, including 191 patients with UTI, 178 patients with non-UTI infections, 50 children with contaminated urine samples, 26 participants with asymptomatic bacteriuria and 75 healthy controls were included in the study. Urine and serum levels of sTLR4, sTLR5 and IL-8 were measured at presentation in all patients and after antibiotic treatment in patients with UTI.

Results Urine sTLR4 was higher in the UTI group than in the other groups. UTI may be predicted using 1.28 ng/mL as cut-off for urine sTLR4 with 68% sensitivity and 65% specificity (AUC = 0.682). In the UTI group, urine sTLR4 levels were significantly higher in pyelonephritis than in cystitis ($p<0.0001$). Post-treatment urine sTLR4 levels in the UTI group were significantly lower than pre-treatment values ($p<0.0001$).

Conclusions Urine sTLR4 may be used as a useful biomarker in predicting UTI and subsequent pyelonephritis in children with UTI.

Keywords Toll-like receptor 4 · TLR4 · Urinary tract infection · UTI · UTILISE study

Introduction

One of the most common bacterial infections in childhood is urinary tract infection (UTI). Urine culture is essential for the diagnosis of UTI and results can be obtained 24–48 h after urine sample collection [1, 2]. Due to the time elapsed until the culture result arrives, the diagnosis and initiation

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of antibiotics are delayed. In order to avoid treatment delay, empirical use of antibiotics is usually prescribed. In a recent study, when empiric antibiotic treatment was initiated in children with suspected UTI, 84% of the urine cultures revealed negative results after 24–48 h, thus antibiotic treatment was ceased [3]. Bacterial contamination of urine culture material or asymptomatic bacteriuria (ABU) is also common in childhood.

A biological marker (biomarker) is measurable in human tissues, cells, or fluids [4] and can be very useful to differentiate real UTI from other infections, contamination and ABU. With the help of this biomarker antibiotic therapy initiation for the patients who have real UTI can be fast and accurate.

In our opinion, a reliable biomarker for UTI should be a part of the host's immune response to UTI. The innate and subsequently adaptive immunity are activated by the entrance of pathogenic microorganisms into the human's urinary tract. Toll-like receptors (TLRs) recognize specific pathogenic agents, subsequently expression of TLRs increases as a response to infectious agents [5–7]. Interferons, chemokines, antimicrobial substances, interleukins and pro-inflammatory cytokines are released by increased expression of TLRs [5–7]. TLRs are expressed in many types of immune cells such as different subsets of dendritic cells, T cells, neutrophils, eosinophils, mast cells, macrophages, monocytes, and epithelial cells [8]. The TLR family has 10 subtypes in humans which are expressed on peripheral blood cells as well as kidney epithelial cells, mesangial cells and bladder [9]. Animal studies demonstrated that mice which are TLR4 and TLR5 deficient cannot respond to microorganisms in their urinary tract properly [10, 11]. Also, it has been shown that soluble forms of TLRs exist in body fluids such as serum, amniotic and cerebrospinal fluid [12–14]. Activation of TLR4 stimulates production of interleukin-8 (IL-8), a potent neutrophil chemoattractant. It has been known that IL-8 has an important role in both mucosal and systemic response to gram negative bacterial infection [15]. This study was conducted as a part of UTILISE (**U**rinary **T**ract **I**nfection and **L**evels of heat shock protein 70 **I**n children as a **S**ensitive marker for **E**xcluding other infections) study [16]. In the UTILISE study, a prospective, multicenter and observational study, it was reported that urine Heat Shock Protein 70 (uHSP70) can be a novel non-invasive biomarker to distinguish UTI from other infections and conditions. uHSP70 is a member of the Heat Shock Protein family. This protein family is synthesized during infections in order to protect the protein structure of the cells [17].

Our aim was to determine whether serum and urine soluble TLR4 (sTLR4), soluble TLR5 (sTLR5) and IL-8 are eligible to be used as biomarkers for the diagnosis of UTI. Also, we aimed to reveal the relationship between uHSP70, which was studied at the first part of UTILISE study, and those biomarkers evaluated in this study.

Materials and methods

Participants from thirty-seven centers and seven countries were included in the study. For Turkish centers, Ethical Committee approval was received from Istanbul University Istanbul Faculty of Medicine Ethical Committee (2017/752). Local investigators applied for the approval of the respective local Ethical Committees for each participating country and the required documents were provided by the principal investigators.

Study protocol

Eligibility of each of a total of 802 children from 37 centers was assessed by two blinded researchers. The ones who did not fulfill the criteria, 282 individuals, were excluded from the study. The remaining 520 children between the ages of 0–18 years were included in the study. A total of 191 patients with UTI was compared with the 75 healthy controls and patient control groups including 178 patients with non-UTI infections, 50 children with contaminated urine samples, and 26 participants with asymptomatic bacteriuria (Supplementary Fig. 1).

Urine sampling for bacterial culture was performed according to the local center protocol (mid-stream/collecting bag/catheter). Sample bags paired with the standard patient information form and labeled sample tubes were prepared for each patient and were sent to the participating centers. Pre-treatment serum and urine samples were obtained before the initiation of antibiotics. Post-treatment samples in patients with UTI were collected after the clinical and laboratory findings improved and antibiotic treatment was ended. Bags for post-treatment samples were prepared only for the UTI group.

Patients

Routine physical examination, serum urea, creatinine (cr), C-reactive protein (CRP), white blood cells measurements, urinalysis and urine culture were performed on patients with symptoms suggesting UTI. A white blood cell count greater than normal values according to age was defined as leukocytosis [18].

Serum CRP < 5 mg/L and procalcitonin < 0.5 ng/ml were considered normal. Estimated glomerular filtration rate (eGFR) was calculated according to the Schwartz formula [19].

Presence of symptoms suggesting UTI along with positive findings in urinalysis and significant bacterial growth in urine culture were the inclusion criteria in the UTI group [1]. Pyuria or nitrite positivity or leukocyte esterase (LE) positivity in urinalysis was accepted as positive findings for UTI

[1]. For samples obtained via collecting bag and mid-stream urine, the significant bacterial growth in the urine culture was $\geq 10^5$ cfu/mL of a single uropathogen, and for samples obtained via catheterization, it was $\geq 10^4$ cfu/mL [20, 21]. UTI group was divided into two subgroups as pyelonephritis and cystitis. Pyelonephritis was defined by clinical and laboratory findings such as fever ≥ 38.5 °C, loin tenderness and/or rigors, leukocytosis and serum CRP level > 5 mg/L. Pyelonephritis was diagnosed in 102 children (53.4%). The standard UTI protocol of the participating centers was applied for antibiotic treatment and duration.

The non-UTI infection group consisted of the patients with non UTI-infection such as upper respiratory tract infection, lower respiratory tract infection, gastroenteritis, meningitis, etc.

Asymptomatic children with neurogenic bladder who were followed with clean intermittent catheterization (CIC) with ≥ 104 cfu/mL bacterial growth of the same microorganism in two consecutive urine cultures with CIC were included in the asymptomatic bacteriuria group [20, 21].

The contamination group consisted of asymptomatic children with urine collection before voiding cystourethrography for suspected vesicoureteral reflux. These children had a growth of a single microorganism $< 10^5$ cfu/mL or ≥ 2 microorganisms in the urine culture [20, 21].

In the healthy control group, there were healthy children who had no history of acute, chronic illnesses and UTI, also without abnormality in the urinary tract. The healthy control group consisted of children coming to the pediatric and adolescent outpatient clinics for a routine control.

Biomarkers

Serum and urine samples from participating centers were sent on dry ice to the laboratory of Istanbul University Istanbul Faculty of Medicine. Samples were stored at – 80 °C. Before the analysis, they were brought to room temperature and were measured by enzyme-linked immunosorbent assay (ELISA) technique using Human sTLR4 and sTLR5 ELISA Kits (Cat no: E4269Hu and E0374Hu, respectively) purchased from Bioassay Technology Laboratory (BT Lab Biotech Co., Shanghai, China) according to the producer's instructions. The unit of sTLR4 and sTLR5 was ng/mL. The detection and quantification limits were set at 0.022 ng/mL and 0.05 ng/mL, respectively. The intra-assay and inter-assay coefficients of variation (CV) of sTLR4 and sTLR5 were < 8% and < 10%, respectively.

According to the producer's instructions, serum and urine IL-8 were measured by enzyme-linked immunosorbent assay (ELISA) technique using Human IL-8 Platinum ELISA kit (Cat no: BMS204/3TEN) purchased from Affymetrix eBioscience (Bender MedSystems GmbH Campus, Vienna, Austria). The unit of IL-8 was pg/mL. The limits of detection and quantification for IL-8 were set at 2 pg/mL. The intra-assay and inter-assay coefficients of variation (CV) of IL-8 were 6.3% and 8.7%, respectively.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics version 22.0 package program for Windows (IBM Corp., Armonk, N.Y., USA). Results are expressed as medians and interquartile range (IQR) for descriptive data. The normality of the parameters was tested using the Kolmogorov–Smirnov normality test. Non-parametric tests (The Mann–Whitney U, Kruskal–Wallis test) were performed for comparisons between groups. The relations between variables were analyzed using the Spearman correlation test. The Chi-square test or Fisher's exact test were performed for the comparison of qualitative data. A receiver operating characteristic (ROC) curve was constructed according to the comparison of the UTI and other groups to calculate the sensitivity and specificity of the assay for urine sTLR4 at varying cut-off values. In addition, AUC values of urine sTLR4 alone and in combination with urinalysis, urine HSP70 and serum CRP were compared. Values of $p < 0.05$ were considered significant.

Results

The median age did not differ between all groups ($p = 0.108$). More girls than boys were in the UTI, ABU and contamination groups ($p < 0.0001$) (Table 1). A total of 85 patients had eGFR < 90 mL/min/1.73 m^2 in UTI, non-UTI infection, and asymptomatic bacteriuria groups (Table 1). The most common complaints were fever (62%), dysuria (37%), and abdominal pain (35%) in the UTI group (Supplementary Table 1). Urinalysis results are given in Table 1. *Escherichia coli* and *Klebsiella pneumonia* were the 2 bacteria most commonly grown in urine culture (66% and 12%, respectively). Clinical findings, laboratory features and urine culture results in children with UTI are presented in Supplementary Table 1.

Serum sTLR4 and serum IL-8 levels were similar between all groups (p > 0.05). Higher serum sTLR5 level was detected in UTI and also in the non-UTI infection group compared to the other three groups ($p = 0.003$) (Table 1).

Urine soluble TLR4

In the UTI group, pre-treatment urine sTLR4 level was higher than in the other groups ($p < 0.0001$). Moreover, urine sTLR4 level was decreased after treatment in the UTI group ($p < 0.0001$) (Table 1).

Table 1 Comparison of study groups according to demographic data, results of urinalysis, serum CRP and procalcitonin, and serum and urine levels of biomarkers

	UTI group ($n=191$)	Non-UTI infection group ($n=178$)	Asymptomatic bacteriuria group ($n=26$)	Contamination group ($n=50$)	Control group ($n=75$)	*P*
Age (years)	6.4 (3.3–9.0)	6.6 (2.8–10.6)	7.4 (2.5–10.1)	7.5 (4.9–11.3)	7.6 (4.9–10.4)	0.108
% females	75	46	72	77	56	< 0.0001
% eGFR < 90 mL/min/1.73 m^2	23	19	31	0	0	
Serum CRP (mg/L)	8.0 (1.2–61.6)	7.5 (2.0–28.0)	1.8 (0.3–3.2)	1.0 (0.3–2.5)	0.4 (0.3–2.2)	< 0.0001
Serum procalcitonin (ng/mL)	0.03 (0.01–0.13)	0.05 (0.01–0.10)	0.01 (0.01–0.02)	0.01 (0.01–0.02)	0.01 (0.01–0.02)	0.001
Serum soluble TLR4 (ng/mL)	1.47 (0.78–5.33) (pre-treatment) 1.02 (0.70–2.23) [a] (post-treatment)	1.54 (0.76–5.18)	1.37 (0.82–2.02)	1.39 (0.79–3.11)	1.78 (0.89–3.05)	0.926
Serum soluble TLR5 (ng/mL)	11.24 (4.75–40.57) (pre-treatment) 7.25 (4.11–16.17)[a] (post-treatment)	14.05 (5.94–50.01)	7.89 (4.34–24.15)	7.99 (4.50–28.00)	6.85 (2.96–14.34)	0.003
Serum IL-8 (pg/mL)	12.93 (11.05–17.58) (pre-treatment) 12.72 (10.90–15.62)[b] (post-treatment)	13.82 (11.53–20.93)	13.30 (11.67–16.17)	13.65 (10.86–20.81)	14.75 (11.72–20.90)	0.437
% with leukocyturia	86.9	11.2	38.5	0	0	< 0.0001
% leukocyte esterase test positive	83.2	7.3	38.5	0	0	< 0.0001
% nitrite positive	60.2	0	0	0	0	
Urine soluble TLR4 (ng/mL)	1.75 (0.96–2.65) (pre-treatment) 0.83 (0.65–1.93)[a] (post-treatment)	0.87 (0.65–1.94)	0.73 (0.61–0.96)	0.70 (0.64–1.83)	1.11 (0.76–1.49)	< 0.0001
Urine soluble TLR5 (ng/mL)	4.94 (2.99–7.66) (pre-treatment) 3.32 (2.04–5.19)[a] (post-treatment)	4.94 (3.16–7.86)	2.64 (1.73–7.19)	3.89 (1.80–5.82)	4.59 (3.69–6.53)	0.018
Urine IL-8 (pg/mL)	125.47 (43.97–395.91) (pre-treatment) 30.49 (16.68–65.51)[a] (post-treatment)	18.47 (11.63–37.91)	65.00 (35.38–182.44)	26.45 (15.41–50.82)	15.72 (10.91–25.64)	< 0.0001

Data are given as median (interquartile range)

UTI Urinary tract infection, *TLR4* Toll-like receptor 4, *TLR5* Toll-like receptor 5, *IL-8* Interleukin-8

[a] Difference pre-post treatment, $p < 0.0001$

[b] Difference pre-post treatment, $p =$ NS

ROC analysis revealed that the optimal cut-off value for urine sTLR4 to predict UTI was 1.28 ng/mL. Using the cut-off > 1.28 ng/mL for urine sTLR4, the presence of UTI was predicted with 68% sensitivity and 65% specificity (AUC = 0.662) (Table 2). Positive predictive value (PPV) and negative predictive value (NPV) were 53% (95% CI of 49–58) and 77% (95% CI of 73–81), respectively (Fig. 1). The specificity of urine sTLR4 increased to 97% combined with the leukocyte esterase test predicting UTI (AUC = 0.773) (Table 2).

In the first part of the UTILISE study, it was shown that uHSP70 can predict UTI using the cut-off value of 48 ng/mL [14]. uHSP70 according to cut-off > 48 ng/mL alone had 89% sensitivity and 82% specificity (AUC = 0.852). If urine

Table 2 Predictive values of urinalysis, urine sTLR4, urine HSP70, and serum CRP for UTI

	AUC	Sensitivity (%)	Specificity (%)
Urine sTLR4 > 1.28 ng/mL	0.662	67.5	64.9
Urine HSP70 > 48 ng/mL	0.852	88.5	81.9
Urine sTLR4 > 1.28 ng/mL and urine HSP70 > 48 ng/mL	0.782	59.7	96.6
Leukocyte esterase test and urine sTLR4 > 1.28 ng/mL	0.773	58.0	96.6
Nitrite test and urine sTLR4 > 1.28 ng/mL	0.712	42.5	100
Leukocyte esterase test and urine sTLR4 > 1.28 ng/mL and urine HSP70 > 48 ng/mL	0.741	48.2	100
Nitrite test and urine sTLR4 > 1.28 ng/mL and urine HSP70 > 48 ng/mL	0.688	37.7	100
Urine sTLR4 > 1.28 ng/mL and urine HSP70 > 48 ng/mL and serum CRP > 5 mg/L	0.669	35.0	98.8

sTLR4 soluble Toll-like receptor 4, *HSP70* Heat shock protein, *CRP* C-reactive protein, *AUC* Area under the ROC curve

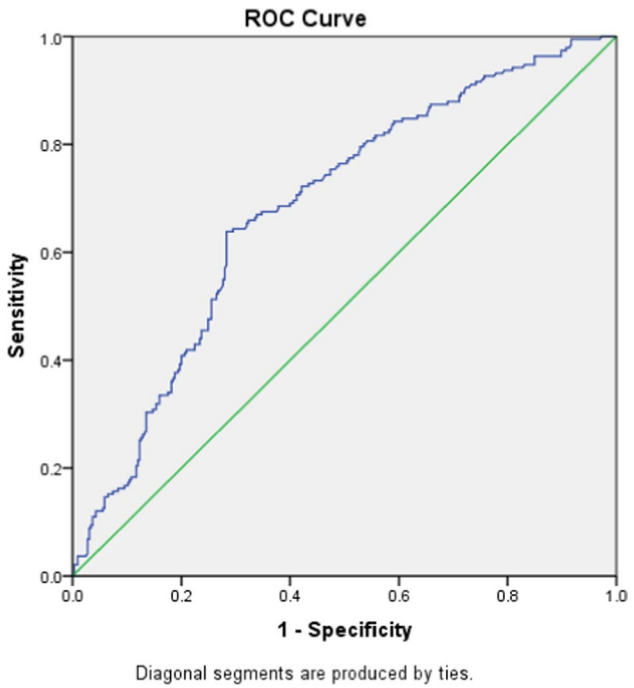

Fig. 1 Receiver operating curve (ROC) analysis showed that the accuracy (AUC) of urine sTLR4 using 1.28 ng/mL cut-off on predicting urinary tract infection was 0.682 (sensitivity 68%, specificity 65%, PPV 53% and NPV 77%)

sTLR4 > 1.28 ng/mL and uHSP70 > 48 ng/mL were used together to predict UTI, the sensitivity and the specificity were respectively 60% and 97% (AUC = 0.782) (Table 2).

In the UTI group, urine sTLR4 level was higher in pyelonephritis than in cystitis (1.88 ng/mL vs 1.47 ng/mL, respectively) ($p < 0.0001$). Using a cut-off 1.69 ng/mL to distinguish pyelonephritis from cystitis, the sensitivity and specificity of urine sTLR4 were 63% and 61%, respectively (AUC = 0.652).

Urine sTLR4 was independent of age ($r = -0.027$ $p = 0.712$) and gender (p = 0.820). Urine sTLR4 was not different between the patients with eGFR ≥ 90 and < 90 mL/min/1.73 m^2 ($p = 0.089$). There was no relationship between urinalysis findings and urine sTLR4 ($p > 0.05$) (Table 3). The patients with UTI caused by other bacteria had significantly higher urine sTLR4 levels than in those with UTI caused by *E. coli* ($p = 0.031$) (Table 3).

Table 3 The relationship of urine sTLR4 levels with urinalysis and urine culture

	Urine sTLR4 (ng/mL)	*p*
Nitrite (+)	1.78 (0.88–2.70)	0.602
Nitrite (-)	1.71 (1.02–2.66)	
Pyuria in microscopy (+)	1.73 (0.96–2.65)	0.933
Pyuria in microscopy (-)	1.76 (0.85–2.49)	
Leukocyte esterase (+)	1.75 (0.90–2.68)	0.773
Leukocyte esterase (-)	1.65 (0.94–2.48)	
E.coli (+)	1.68 (0.87–2.49)	0.031
E.coli (-)	1.90 (1.12–3.44)	

Data are given as median (interquartile range)

sTLR4 soluble Toll-like receptor 4, *E.coli Escherichia coli*

Urine soluble TLR5

Pre-treatment urine sTLR5 level was lower in the ABU group than in all other groups ($p = 0.018$) (Table 1). Urine sTLR5 level was not significantly higher in the UTI group, thus sensitivity and specificity were not calculated.

Urine IL-8

Pre-treatment urine IL-8 level was higher in the UTI and ABU groups than in all other groups ($p < 0.0001$). Although urine IL-8 median level was higher in the UTI group than in the ABU group, the difference between these two groups was not statistically significant ($p = 0.067$). Urine IL-8 level

did not differ between pyelonephritis, cystitis and ABU ($p > 0.05$). Post-treatment urine IL-8 level in the UTI group was lower than pre-treatment values ($p < 0.0001$) (Table 1).

Discussion

In this prospective study, we showed that urine sTLR4 levels increased in UTI and it was higher in pyelonephritis than in cystitis. Moreover, elevation of urine sTLR4 in these patients was certainly due to UTI since high urine sTLR4 levels at the onset of UTI significantly decreased after antibiotic therapy. Therefore, it is possible to follow the response to treatment by consecutive urine sTLR4 measurements. Another important finding about urine sTLR4 was that the levels were not affected by gender, age and eGFR.

There are various studies investigating the relationship of genetic polymorphisms regulating functions of TLR4 with susceptibility to UTI. It has been reported that polymorphisms in the TLR4 genes *Asp299Gly* (+ 896A > G, rs4986790) and *Thr399Ile* (+ 1196C > T, rs4986791) increase susceptibility to UTI [22–25]. Karananou et al. [26] noted that the children who had experienced one or more episodes of acute UTIs had higher expression of TLR4 on monocytes than in children who had no previous UTI history. Bayram et al. [27] also found higher *Thr399Ile* polymorphism, yet lower TLR4 expression on monocytes and neutrophils in patients with febrile UTI compared to healthy controls. Based on these studies, we hypothesized that UTI episodes may induce higher TLR4 expression and we were able to show the relationship between urine sTLR4 levels and UTI.

Our results suggest that urine sTLR4 might contribute to predict UTI, if its level is > 1.28 ng/mL. However, urine sTLR4 could not be shown to be superior in accuracy of urinalysis as a well-known diagnostic test for predicting UTI. Urine sTLR4 does not have higher sensitivity and specificity than urinalysis parameters already shown in previous studies [1]. It was not possible to compare the urine sTLR4 with urinalysis in our study since one of the inclusion criteria for the diagnosis of UTI was LE or nitrite test positivity. Therefore, we analyzed the combination of urine sTLR4 and findings of urinalysis. Our findings showed that the specificity of urine sTLR4 increased to 97% in combination with LE, yet the sensitivity decreased. The combination of both LE and urine sTLR4 may be used as a parameter to exclude UTI due to high specificity.

As the discovery of new non-invasive markers for the early detection of UTI is an interesting topic, there are numerous studies evaluating various candidate biomarkers such as neutrophil gelatinase-associated lipocalin (NGAL), interleukins (IL), heparin binding protein (HBP), lactoferrin (LF), heat-shock protein-70 (HSP-70), human defensin-5 (HD-5), and lipopolysaccharide binding protein (LBP) [28]. We have also previously shown that urinary neutrophil gelatinase-associated lipocalin (NGAL) and HSP70 can be used as biomarkers to predict UTI [16, 29]. It has been reported in many observational, comparative studies that NGAL has a leading role in the diagnosis and differentiation of UTIs [28–30]. Since NGAL is already a promising biomarker, we did not include it in our present study and evaluated the role of other biomarkers that may contribute to predict UTI. Although the sensitivity and specificity of urine sTLR4 was not as high as NGAL, we obtained data to guide further research.

In the first part of the UTILISE study [16] it was demonstrated that uHSP70 level had a better AUC value than urine sTLR4 to predict UTI. However, combination of urine sTLR4 and uHSP70 increased the specificity up to 97%, while simultaneously the sensitivity decreased. This combination provides no additional benefit over the use of LE and urine sTLR4 together. uHSP70 predicts UTI with high sensitivity and specificity, yet not pyelonephritis. Ability to distinguish pyelonephritis from cystitis may be an advantage of urine sTLR4 over uHSP70. Urine sTLR4 may be used to distinguish pyelonephritis with cystitis in patients with UTI diagnosed according to findings of urinalysis or uHSP70 > 48 ng/mL.

The distinction between pyelonephritis and cystitis is mostly based on clinical findings and laboratory results. Nevertheless, this distinction may not always be so easy, especially in infants. For instance, febrile cystitis can also be detected in some children [31]. An accurate biomarker such as urine sTLR4 might non-invasively guide the differentiation of pyelonephritis and cystitis in children.

Many studies have shown that urine IL-8 was elevated in patients with UTI compared to healthy children [32–34]. Similarly, in our study, urine IL-8 activated by TLR4 was also higher in UTIs than in healthy children, yet it did not distinguish UTI from ABU. Benson et al. [35] noted that urine IL-8 was significantly higher in febrile UTI than in an ABU group. Krzemien et al. [36] reported that urine levels of IL-8 were higher in febrile UTI compared to non-febrile UTI and ABU in children between 1–12 months. In the same study, no significant differences in cytokine levels were documented between children with non-febrile UTI and ABU. Contrary to these studies, we did not find any difference in urine IL-8 levels between the ABU, pyelonephritis and cystitis groups. We concluded that the difference between the findings of two studies mentioned above [35, 36] may be due to patient age. The mean ages of their participants were younger than our study. Moreover, the ABU group in our study consisted of only children with neurogenic bladder who receive CIC. Chronic inflammation may occur in children receiving CIC, and cytokines may play an active role in chronic inflammation

[37]. That may influence the interpretation of urine IL-8 in the UTI and ABU groups in our study.

The limitation of our study was that a dimercaptosuccinic acid (DMSA) scan was not performed to differentiate acute pyelonephritis from cystitis. Patients were classified as having pyelonephritis based on clinical and laboratory findings.

In conclusion, our findings suggest that urine sTLR4 has a role in the immune response to UTI. Urine sTLR4 levels may help to predict UTI in children yet AUC values were lower than that of uHSP70 alone. However, urine sTLR4 level has been shown to be a better predictive value to distinguish pyelonephritis with cystitis. By using uHSP70 or LE and urine sTLR4 consecutively we determined higher specificity. Therefore, adding a different biomarker with high sensitivity might predict UTI more accurately. Further studies are needed to discover new biomarkers to predict UTI.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00467-023-06063-0.

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Declarations

Conflict of interest disclosure The authors have no conflicts of interest to disclose.

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