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ORIGINAL ARTICLE

Urine flow cytometry is an adequate screening tool for urinary tract infections in children

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Abstract

Diagnosing a urinary tract infection in children is often difficult due to non-specific symptoms and requires invasive and time-consuming procedures. Flow cytometry is a new and rapid method of analyzing urine to confirm or exclude UTIs. We have investigated the sensitivity and specificity of urine flow cytometry (Sysmex UF1000i) compared to conventional diagnostic techniques in a prospective study from January 1, 2014 until January 1, 2015. All children under 13 years of age with a suspicion of urinary tract infection were screened using both urine flow cytometry and urine culture. A urinary tract infection was defined as the combination of leukocyturia (≥ 25 leukocytes per μl) and a positive urine culture in the presence of clinical symptoms. A total number of 412 urine samples were collected, of which 63 cases (15.3%) were positive for a urinary tract infection. Receiver operating characteristic analysis showed an area under the curve of 0.97 (95% confidence interval h0.93–1.00) for the bacterial count. When using a cut-off value of 250 bacteria/μl in the presence of leukocyturia, the sensitivity for urinary tract infection is 0.97 with a negative predictive value of 97%, and the specificity is 0.91 with a positive predictive value of 90%.

Conclusion: Flow cytometry–based bacterial and leukocyte count analysis is a time-efficient method of diagnosing or ruling out urinary tract infection in children, with a higher sensitivity and specificity than dipstick and microscopic analysis.

What is known

- *Screening for urinary tract infections in children is difficult due to invasive and time-consuming procedures.*
- *There is both over- and under-treatment of urinary tract infections due to the delays in accurate diagnosing.*

What is new

- *Flow cytometry is a rapid and accurate method to provide useful information in the diagnosis of urinary tract infection in children. When negative, flow cytometry can exclude urinary tract infection in children with a high degree of confidence. When flow cytometry is positive, the possibility of a urinary tract infection in children is increased.*

Keywords Urinary tract infection · Urine flow cytometry · Children · Screening

Maarten Broeren and Rélana Nowacki contributed equally to this work.

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Abbreviations

AUC Area under the curve
CFU Colony forming units
CI Confidence interval
ROC Receiver operating characteristic
UTI Urinary tract infection

Introduction

Urinary tract infections (UTIs) are common in children; the estimated prevalence is 3–7% for children with fever of unknown origin below 2 years of age [9, 11, 20] and up to 14% in infants aged 8 weeks and younger [15]. UTIs may have serious complications; renal scarring is visible on dimercaptosuccinic acid (DMSA) scintigraphy in up to 86% of children immediately after a UTI which may lead to impaired renal function [2, 12]. Especially in young children, the diagnosis of a UTI is difficult to make due to non-specific symptoms such as fever and vomiting, and often requires invasive and time-consuming procedures. Because delay in treatment can lead to serious complications including sepsis and renal damage [6], and overtreatment on the other hand causes unnecessary side effects and may contribute to bacterial resistance to antibiotics, it is important to adequately identify or rule out a UTI as soon as possible.

When a UTI is clinically suspected, the gold standard for confirming the diagnosis is a positive urine culture that exceeds a quantitative growth threshold in the presence of leukocyturia, but the time to results can be 1–2 days. To rapidly screen for a UTI, several methods have been developed. The most widely used methods are the dipstick urinalysis and urine microscopic analysis. Dipstick urinalysis indicates the presence of urinary nitrite and leukocyte esterase as a surrogate marker for leukocyturia [21]. However, its sensitivity and specificity are limited, and the bacterial count is not included in the screening. The other frequently used method is microscopic urinalysis for leukocyte and bacterial count. The average sensitivity and specificity for leukocytes in this test are 0.73 and 0.81 and for the bacterial count 0.81 and 0.83 respectively [21]. Thus, the currently most used screening methods lack sufficient sensitivity and specificity in children, with poor sensitivity risking missing UTIs which may have detrimental sequelae, and poor specificity leading to unnecessary antibiotic overtreatment, with the risk of side effects and increasing bacterial resistance to antibiotics.

The Sysmex UF1000i urine flow cytometer rapidly quantifies elements in urine such as erythrocytes, leukocytes, and bacteria, and is a relatively new instrument to evaluate suspected UTIs [4]. Flow cytometry results can be available within 10 to 30 min. Several studies have already been performed to set the reference values for leukocyte and bacterial counts in adults [3, 4, 8]. Our aim was to investigate the sensitivity and specificity of urine flow cytometry–based bacterial and leukocyte counts to assist with the diagnosis of urinary tract infections in a general pediatric population.

Materials and methods

This prospective study was conducted from January 1, 2014 until January 1, 2015. During this period, all pediatric patients 0–13 years of age, including preterm infants, with a suspected UTI seen in either the emergency room, during hospitalization, or in the outpatient clinic of the Máxima Medical Center, Veldhoven, the Netherlands, were screened. Clinical symptoms indicative for UTI included fever, abdominal pain, back pain, or urinary symptoms like dysuria, frequency, urgency, or incontinence. Samples were excluded from analysis (1) when obtained using a collection bag, (2) during or within 48 h after cessation of antibiotic therapy to avoid possible false-negative urine cultures, or (3) when they contained incomplete data or testing. From all included patients, two aliquots of urine were collected and stored in a fridge while awaiting processing. All samples were processed within 4 h by both flow cytometry and urine culture.

One aliquot was used for flow cytometry which was performed by the Sysmex UF1000i Automated Urine Particle Analyzer (Sysmex America Inc., Lincolnshire, IL, USA). The UF1000i quantifies the formed elements to the nearest 0.1 cell per milliliter (ml) in 0.8 to 1.2 ml of uncentrifuged urine based on size, shape, and staining characteristics [19]. The minimal detection threshold for leukocytes in the UF1000i was set to 25/μl, which represents the advised threshold of 5 leukocytes per high-power field when using microscopy on centrifuged urine [21]. Leukocyte concentrations below 25/μl were reported as $< 25/\mu l$.

The other aliquot was used for Gram stain and urine culture. Gram stains were analyzed by a trained technician who scored the presence of epithelial cells, leukocytes, yeasts, and bacteria, with the morphology on a scale rating from absent to 4+. Ten microliters of each sample was inoculated on a chromogenic medium (Brilliance UTI clarity agar, OXIOD) and a selective blood agar plate containing 5 μg/ml colisitin and 2 μg/ml aztreonam. Both plates were investigated for growth after 18 to 24 h of aerobic incubation at 37 °C. Based on preset validated threshold values, the amount of growth was scored as no growth, 10^3 to 10^4 colony forming units/ml (CFU/ml) growth, 10^4 to 10^5 CFU/ml growth, and $\geq 10^5$ CFU/ml growth. Most grown uropathogens were identified by their color: pink to red (*Escherichia coli*), brown halo (*Proteus mirabilis*), turquoise to blue-green, and growth on CAP blood agar (*Enterococcus* spp.). The

remaining colonies were identified by MALDI-TOF MS (Bruker). A urine culture was considered positive when a single urinary pathogen was present of more than 10^4 CFU/ml when the sample was obtained by catheterization or more than 10^5 CFU/ml when obtained by midstream or clean catch technique. This definition is according to the American Association of Pediatrics guidelines for UTIs in children [1, 10, 21]. If a urine culture showed multiple pathogens in a concentration of $\geq 10^4$ or $\geq 10^5$ CFU/ml depending on the collection method, a diagnosis of UTI was made on the doctors' discretion, based on the clinical history and the species of the pathogens.

A UTI was defined as the combination of flow cytometry leukocyturia (≥ 25 leukocytes per μl) and a positive urine culture. Samples that were negative for leukocyturia based on flow cytometry were classified as UTI-negative, regardless the outcome of the urine culture. In the presence of leukocyturia, the bacterial count using flow cytometry was compared to urine culture.

To determine cut-off values for urine flow cytometry as a screening method, in general, it is stated that 100 samples are required [18]. Because of the varying prevalence numbers of UTIs, we set the prevalence to 7.5% based on prevalence numbers reported in literature and prevalence estimates in the Netherlands [1, 11, 20]. We calculated a sample size of 387 patients to yield 95% confidence intervals (CI) 10% around a point estimate for a sensitivity of 0.92.

Data analysis was performed using Microsoft Excel (Microsoft Office Professional Plus 2010). Receiver operating characteristic (ROC) curves were made using Analyse-it (version 2.22, Analyse-it Software, Ltd., Leeds, United Kingdom). ROC curves were created to asses sensitivity and specificity of cut-off values of both leukocyte and bacterial count. The area under the curve (AUC) was used to investigate the ability of leukocyte and bacterial count to predict a UTI. The difference in medians for bacterial and leukocyte counts in the UTI-positive and UTI-negative populations was analyzed using a Wilcoxon-Mann-Whitney test. A subanalysis was performed to evaluate the influence of urine samples containing growth $\geq 10^5$ CFU/ml and multiple pathogens on flow cytometry test characteristics. This was done by recalculation of the AUC of the ROC curve, as well as recalculation of the sensitivity and specificity at a cut-off value of 250 bacteria/μl after exclusion of these samples.

Results

In total, 623 samples were collected from pediatric patients with a suspicion of UTI. Exclusion of samples that were collected using a collection bag ($n = 130$), samples that were obtained during or within 48 h after cessation of antibiotic therapy ($n = 56$), and samples with incomplete data ($n = 25$) resulted in 412 urine samples available for analysis. Of these, 136 (33.0%) were from male patients. The mean age of the patients was 5.3 years, ranging from 2 days of age to 12.9 years. Of all urine samples, 12 samples (3%) were obtained from patients who had a medical history with increased risk for developing complicated UTIs such as hydronephrosis or renal cysts. Patient and urine sample characteristics are displayed in Table 1. Urine culture showed no growth or bacteria seen in the Gram stain in 261 samples (63%), growth of $< 10^4$ CFU/ml in 49 samples (12%), and growth of $< 10^5$ CFU/ml in 23 samples (6%). In 79 samples (19%), bacterial growth of $\geq 10^5$ CFU/ml was observed. Of all samples, 17% contained multiple species. Growth of $\geq 10^5$ CFU/ml containing multiple species was observed in 14 samples. The most common microorganisms identified were *Escherichia coli* (88%), *Citrobacter koseri* (5%), *Enterococcus faecalis* (3%), *Klebsiella* spp. (3%), and *Proteus mirabilis* (1%). Of the 14 samples that showed growth $\geq 10^5$ CFU/ml and more than one urinary pathogen, *Escherichia coli* was identified in 11 samples in combination with other pathogens (mixed flora ($n = 7$), *Enterococcus*

Table 1 Patient and urine samples characteristics

Characteristics	UTI ($n = 63$)	No UTI ($n = 349$)	Total ($n = 412$)
Age, mean years (median/minimum–maximum)	5.3 (5.1/0.0–12.2)	5.3 (5.0/0.0–12.9)	5.3 (5.1/0.0–12.9)
Age category, *n* (%)			
0 to 3 months	6 (9)	43 (12)	49 (12)
3 months to 3 years	15 (24)	52 (15)	67 (16)
3 years or older	42 (67)	254 (73)	296 (72)
Male, *n* (%)	15 (23.4)	121 (34.8)	136 (33.0)
Obtained by midstream/clean catch, *n* (%)	47 (74.4)	305 (87.6)	352 (85.4)
Obtained by catheterization, *n* (%)	17 (26.6)	43 (12.4)	60 (14.6)
Leukocyte count (/μl), median (minimum–maximum)	360 (30–25,810)	< 25 (< 25–11,030)	< 25 (< 25–25,810)
Bacterial count (/μl), median (minimum-maximum)	27,302 (190–100,000)	445 (0–67,500)	4617 (0–100,000)

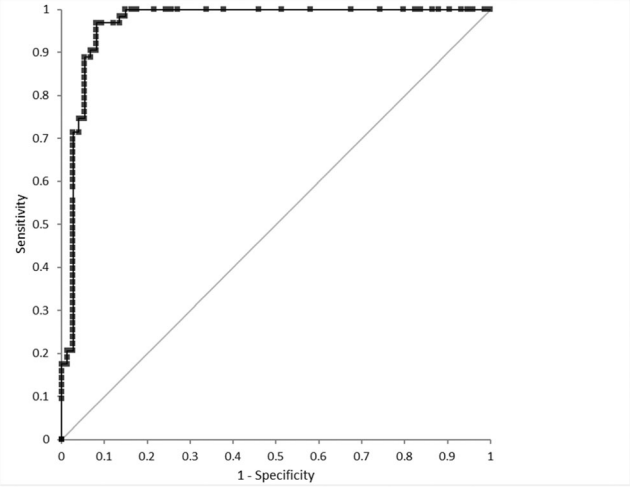

Fig. 1 ROC curve for the bacterial count obtained by urine flow cytometry on leukocyte positive samples ($n = 138$) compared to gold standard urine culture. AUC, 0.97

faecalis ($n = 3$), *Streptococcus agalactiae* ($n = 2$), *Klebsiella oxytoca* ($n = 1$), Gram-negative rods not specified ($n = 1$)). One sample contained both *Citrobacter koseri* and *Enterococcus faecalis*, two samples showed mixed flora not further specified.

A UTI was diagnosed in 63 cases (15.5%), of which 15 (23.4%) were male patients. Of these 63 samples, 62 samples showed bacterial growth of $\geq 10^5$ CFU/ml in the urine culture, of which 12 (19%) samples showed multiple microorganisms. One sample showed bacterial growth of 10^4–10^5 CFU/ml and was collected via a catheter. The median bacterial and leukocyte counts were significantly higher in the group of UTI (13.950 vs. 30 bacteria/μl and 360 vs. < 25 leukocytes/μl respectively, $p < 0.01$ for both parameters) (Table 1). Leukocyturia was present in 137 samples. In these leukocyte positive samples, ROC analysis showed an area under the curve (AUC) of 0.97 (95% confidence interval (CI), 0.93–1.00) for the bacterial count (Fig. 1). Leukocyturia was present in all 14 samples with growth $\geq 10^5$ CFU/ml and multiple species.

Applying variation in cut-off points for the bacterial count resulted in variable sensitivity and specificity (Table 2). For instance, when using a cut-off value of 250 bacteria/μl, the sensitivity is 0.97 with a negative predictive value of 97%, and the specificity is 0.91 with a positive predictive value of 90%. In the samples with multiple pathogens and growth $\geq 10^5$ CFU/ml, leukocyte and bacterial counts were high with median values of 425 leukocytes/μl and 19,650 bacteria/μl. Twelve of these samples were considered positive for UTI. Exclusion of these samples and reanalysis of the data resulted in an AUC of the ROC curve of 0.98 (95% CI, 0.96–1.00), and a sensitivity and specificity at a cut-off of 250 bacteria/μl of 0.96 and 0.90, respectively.

Because some guidelines define a UTI as a positive urine culture regardless of the presence of leukocyturia, we have also compared bacterial count and leukocyte count using flow cytometry to mere urine culture. This resulted in eight more UTIs ($n = 71$). The analysis of this cohort showed an AUC of 0.96 (95% CI, 0.93–0.99) for the bacterial count and an AUC of 0.89 (95% CI, 0.85–0.94) for the leukocyte count (Fig. 2). Applying variation in cut-off points for the bacterial count resulted in variable sensitivity and specificity (Table 3).

Discussion

At a cut-off value for bacteria of 250/μl, we found a sensitivity of 0.97 with a negative predictive value of 97% and a specificity of 0.91 with a positive predictive value of 90% for the detection of UTI on leukocyte positive samples ($n = 137$, Table 2) in urine flow cytometry. Only two false-negative results were obtained, both from a urine sample positive for *Escherichia coli*. Based on the total number of samples ($n = 412$), our results imply that less than 1% of UTIs in children would be missed when using urine flow cytometry as screening method and only 10% would receive unnecessary antibiotic treatment. When leukocyturia is excluded as a criterion for UTI and the gold standard is solely based on a positive urine culture, the numbers are slightly lower (Table 3), a sensitivity of 0.90 with a negative predictive value of 98% and a specificity of 0.93 with a positive predictive value of 72%. Regardless the gold standard for UTI, these results of urine flow cytometry are superior to dipstick analysis and urine microscopy. Hoberman et al. showed a false-negative leukocyte esterase in 47% of the samples with leukocyturia (defined

Table 2 Different cut-off values of urine bacterial count and their sensitivity, specificity, and predictive values for UTI when urine culture is applied as gold standard on leukocyte positive samples

Cut-off	Sensitivity	95% CI	Specificity	95% CI	NPV (%)	PPV (%)
170/μl	1.00	0.94–1.00	0.85	0.75–0.92	100	85
250/μl	0.97	0.89–0.99	0.91	0.82–0.95	97	90
300/μl	0.94	0.85–0.98	0.92	0.83–0.96	94	91
500/μl	0.89	0.79–0.95	0.95	0.87–0.98	91	93
2500/μl	0.80	0.68–0.88	0.95	0.87–0.98	84	93
6000/μl	0.65	0.53–0.76	0.97	0.91–0.99	77	95

NPV negative predictive value, *PPV* positive predictive value, *CI* confidential interval

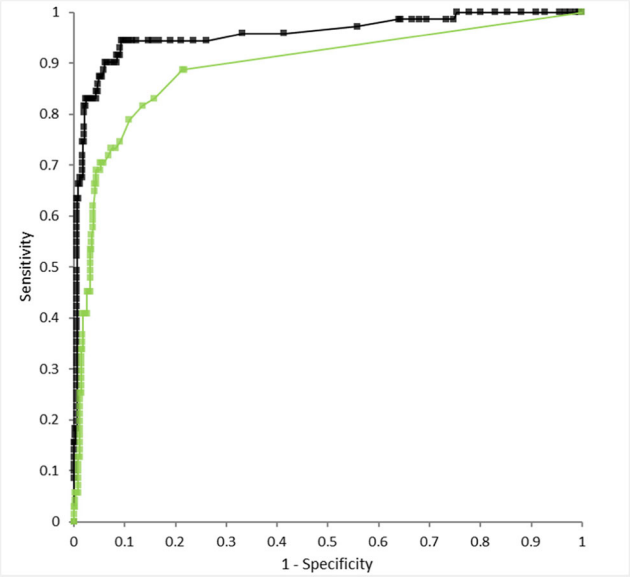

Fig. 2 ROC curves for bacterial count (black) or leukocyte count (green) obtained by urine flow cytometry on all samples ($n = 412$) compared to gold standard urine culture. AUC for bacterial count, 0.96; AUC for leukocyte count, 0.89

as > 10 leukocytes per mm^3) [9]. In the American Association of Pediatrics guidelines [21], the sensitivity for leukocyte esterase detected by dipstick analysis is mentioned to be 0.83 and the specificity 0.78, whereas the sensitivity and specificity for nitrite is 0.53 and 0.98 respectively. Combined, the sensitivity is 0.93 and the specificity 0.72, which is significantly lower than our results with urine flow cytometry. The reduction in unwarranted urine cultures will lead to (1) less unnecessary antibiotic treatments and its possible adverse effects while waiting for culture results, (2) a lower risk of developing bacterial resistance to commonly prescribed antibiotics, and (3) possibly lower health care costs.

This study is among the first to set cut-off values for the sensitivity and specificity of urine flow cytometry using the Sysmex UF-1000i in children; most other studies have been performed in merely adults or in a mixed cohort of adults and a small number children [7]. Also, previous studies did not use urine cultures or clinical symptoms to correlate with the results obtained with flow cytometry [8, 17]. One previous study has described the efficacy of flow cytometry in young children presenting to the emergency department but included only patients under 4 years of age [13], while we have studied a larger cohort with inclusion of patients aged 0 to 13 years, from a pediatric population in a general hospital presenting with symptoms possibly due to a UTI.

In our study, the bacterial count appeared to be the best parameter for predicting a UTI (Fig. 2). This is in accordance with other studies in adults and children that use the Sysmex UF-1000i flow cytometer [4, 5, 13, 14], but in contrast to a recent study by Duong and coworkers who found that the leukocyte count gave the best diagnostic performance in screening for UTI in children [7]. However, they use the Sysmex UF-100 flow cytometer, which is less advanced in detecting bacteria in urine [5]. The prevalence of a UTI in our study (15.5%) was higher than in most previous studies; however, the reported prevalence numbers range widely depending on cohort characteristics of the studied populations, ranging from 2% in afebrile girls to 21% in uncircumcised boys [16, 22]. In our study, the indication for urine investigations was made by the attending pediatric physician, representative for a general practice and diagnostic work-up in most hospitals. The a-priori probability of a UTI might be slightly increased in our study cohort due to the inclusion of 12 samples from patients with prior medical history of renal cysts or hydronephrosis, with known higher prevalence of UTI. Another reason for the higher prevalence may be our definition of a UTI; we have included 14 samples that showed culture growth with more than one pathogen, which may have been considered contamination in other studies. In our study, however, we considered these as positive for UTI when clinical symptoms and leukocyturia were present, and the CFU for each bacteria was $\geq 10^4$/ml or $\geq 10^5$ CFU/ml depending on sample collection ($n = 12$). Over half of these samples ($n = 7$) also had a positive nitrite test, which is strongly indicative for a UTI with a specificity of 0.98 in symptomatic patients [21]. All samples ($n = 14$) were also positive for UTI based on urine flow cytometry, as the bacterial count was high (median, 19650/μl). Exclusion of these samples barely influenced the AUC of the ROC curve for the bacterial count, as well as the sensitivity and specificity.

Table 3 Different cut-off values of urine bacterial count and their sensitivity, specificity, and predictive values for UTI when urine culture is applied as gold standard

Cut-off	Sensitivity	95% CI	Specificity	95% CI	NPV (%)	PPV (%)
170/μl	0.94	0.87–0.98	0.91	0.87–0.98	99	68
250/μl	0.90	0.81–0.95	0.93	0.89–0.95	98	72
300/μl	0.87	0.78–0.93	0.94	0.92–0.96	97	77
500/μl	0.83	0.73–0.90	0.97	0.94–0.98	96	83
2500/μl	0.70	0.59–0.80	0.98	0.96–0.99	94	89
6000/μl	0.58	0.46–0.69	0.99	0.98–1.0	92	95

NPV negative predictive value, *PPV* positive predictive value, *CI* confidential interval

Conclusion

Urine flow cytometry using the Sysmex UF-1000i is a time-efficient method in diagnosing or ruling out UTI in the heterogenic group of pediatric patients in a general hospital. The bacterial count is the most sensitive and specific marker for predicting a UTI in symptomatic patients with leukocyturia, with higher sensitivity and specificity than any known dipstick and microscopic analysis.

Authors' contributions M. Broeren participated in the study design analysis and especially the statistical analysis, and writing the manuscript.

R. Nowacki participated in the study design analysis, patient inclusion and writing the manuscript.

F. Halbertsma participated in the study design, patient inclusion, analysis and writing the manuscript.

N. Arents participated in the micribiological analysis, and writing the manuscript.

S. Zegers participated in the study design analysis the statistical analysis, and writing the manuscript especially related to clinical relevance.

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