Correlation of Urine Leukocytes and Urine Bacteria Using Current Sitometry Using Urine in Patients of Children Channel Infection

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Abstract

Background: Urinary tract infection (UTI) in children is a common illness. The gold standard for UTI diagnosis is urine culture, but it takes a long time and often gives negative results. Currently developed a method of flow cytometry for checking urine sediments. This tool is able to detect leukocytes urine and urine bacteria in quick time. The purpose of this study is to analyze the relationship of urine leukocyte count and the number of urine bacteria using flow cytometry with urine culture in patients with UTI

Method: Eighty-four patients were enrolled in this study that consisting of 43 patients with suspected UTI and 43 patients with non-ISK treated at Inpatient Installation, Emergency Installation Department of Health Sciences at Dr.Soetomo General Hospital, Surabaya for 4 months. The examination of urine leukocytes and urine bacteria was using current cytometry method (UF-500i, Sysmex, Japan). While, examination of urine culture using blood agar plate and Mac. Conkey.

Results: There was a significant relationship between the number of leukocytes and the number of colonies. The relationship between leukocytes and the number of colonies was weak, this was indicated by Spearman correlation coefficient (rho) value of 0.376. There was a significant relationship between bacteria and the number of colonies. $p < 0.05$. The relationship between the number of urine bacteria and the number of colonies was quite strong indicated by rho = 0.729.

Conclusion: There was a relationship between urine leukocyte count and the number of urine bacteria using flow cytometry with a urine culture.

Keywords: *Urine leukocytes, Urine bacteria, Urine culture, Urinary tract infections.*

Introduction

Urinary tract infection (UTI) in children is a common illness that found in addition to upper respiratory tract infections and diarrhea and the second most common cause of fever after upper respiratory tract infection [1]. Urine culture examination takes a long time and can give false negative results. It may delay the diagnosis of UTI, which in the end the management of UTI is also delayed, which may lead to poor UTI prognosis. The incidence of UTI in children at Indonesia ranges from 0.1% to 1.9% of all cases that children treated within 5 years (1984-1989) [2].

The high incidence of UTI causes many requests for urine culture examination[3,4]. Positive urine culture when bacterial growth of more than 100.000 colony forming units (CFU)/mL from one pathogen[5]. 60 - 80% urine culture examination did not contain any infectious bacteria or just contaminants that can be solved by filter test [4,6,7]. Examination of bacteria with Gram staining (sensitivity 96%, specificity 93%), examination using 10 cell/µL hemositometer (83% sensitivity and specificity) [8]. Microscopic examination can give results faster than culture, more simple and inexpensive. But

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the results of the examination require skilled, trained, and interpretation among interrogators can be different [6]. In addition to microscopically, examination of urine and urine leukocyte bacteria can use the method of flow cytometry. Current cytometry methods can distinguish particles in urine and quantitatively calculate them, including leukocytes, erythrocytes, epithelium, bacteria, fungi, cylinders, and crystals. Sysmex UF-500i is one example of urine sediment examination tool using current cytometry method. This tool is able to check large quantities of samples with fast time [6,9,10]

The sensitivity and specificity of the examination using flow cytometry to confirm the diagnosis of UTI is non-existent. Previous studies have suggested that the 65-bit bacteria/mL and 100 leukocyte/mL cutoff points gave 98% sensitivity, specificity 62.1%, negative predictive value 98.7%, positive predictive value 53.7% [11]. Other studies also stated that at the point of cutting of 230 bacteria/μL, urine culture <105 CFU/mL obtained 95% sensitivity and 80% specificity and able to reduce the number of urine culture examination by 52% [9]. Research using flow cytometry in Indonesia is still rare. In this research will be examined using flow cytometry to assess the relationship of urine and urine leukocytes with urine culture results.

Method

This research was an observational analytic research with the cross-sectional design . The research started from the literature search, research proposal preparation, sample collection and workmanship, data management, and research report preparation conducted from March until June 2013. The research was conducted at Inpatient Installation, Outpatient Installation and Emergency Installation of Department Child Health Science Dr.Soetomo General Hospital/Faculty of Medicine Universitas Airlangga Surabaya, as the place of sampling. Sample examination was performed at the Clinical Pathology Installation of Dr.Soetomo/Faculty of Medicine Universitas Airlangga, Surabaya. The sample of the study was the pediatric patient and divided into two groups: groups of patients with UTI and Non-UTI that meet the inclusion criteria.

The inclusion criteria for samples meeting the criteria in this study included patients with suspected UTI aged 2 to 18 years, patients receiving antibiotics, doing urinalysis examination and obtaining positive esterase leukocyte, positive nitrite, uric microstatic leukocyte> 5/Lpb or found urine bacteria microscopic, and willing to sign informed consent. Urinalysis results obtained negative nitrite, leukocyte esterase negative, leukocyte urine microscopic <5Lpb or not found microscopic urine bacteria.

This study was started by selecting the sample according to the inclusion criteria, then the urine sampling was performed by catheterization in children less than 6 years old and the way of urine transmit clean in children more than 6 years old. After the urine sample is directly sent to the Installation of Clinical Pathology Dr.Soetomo General Hospital for urine examination using flow cytometry (UF 500i) (Sysmex Corporation, Japan) and urine culture was done within ≤ 1 hour.

All collected data collected in the data collection sheet was presented in tabular form, the diagram was processed statistically in the form of descriptive analysis. To calculate sensitivity, specificity, positive predictive value, negative and ROC curve, and to know the relation of urine leukocyte count and urine bacteria, statistic analysis test using Pearson correlation test if the data was normally distributed if the data not normally distributed using Spearman correlation test.

Results

Table 1. The suitability between the tilt image on the flow cytometry and the bacterial form of the urine culture

Tilt picture	n	Right	%
Tilt 1	3	2	66,.7
Tilt 2	15	14	93.3
Total	18	16	88.9

Table. 2 Table 2 x x2 examination of urine leukocyte count and amount of urine bacteria using flow cytometry

	Urine Culture ≥ 5 x 104 CFU/mL			
	UTI		Non-UTI	
	Positive	Negative	Positive	Negative
Urine Leukocytes ≥ 40/µL	17	9	11	49
Urine bacteria ≥ 125/µL	18	8	3	57

Table. 3 The diagnostic value of urine leukocyte examination using urine flow cytometry

	Urine Leukocytes		**Urine Bacteria**	
Diagnostic value		CI (95%)		CI (95%)
Diagnostic sensitivity (%)	65.4%	44.4 – 82.1	69.2%	48.1 – 84.9
Diagnostic specificity (%)	81.7%	69.1 – 90.1	95.0%	85.2 – 98.7
Positive predictive value (%)	60.7%	40.7 – 77.9	85.7%	62.6 – 96.2
Negative predictive value (%)	84.5%	72.1 – 92.2	87.7%	76.6 – 94.2
Possibly positive ratio	3.6	1.9 – 6.5	13.8	4.5 – 42.9
Possibly negative ratio	0.4	0.2 – 0.7	0.3	0.2 – 17.3

The results of the examination of the number of urine bacteria obtained the lowest value of 0.0/µL and the highest 58017.8/µL. Median examination results of urinary bacterial counts in the UTI group of 671,85/ µL with a range of 19.4/µL to 58017.8/µL. Median examination results of urinary bacterial counts in the non-UTI group were 7.2/µL with a range of 0.0/µL to 832/µL. There was a significant difference in the number of urine bacteria between group UTI groups rather than UTI, $p < 0.05$ (Figure.4B). Current cytometric examination can also display a scattergram image that can be viewed on scattergram B1 (Figure.3BA). Urine bacteria is represented by a purple (purple) color. There are two typical features of the slope of the bacterial scattergram namely the inclination 1 (Figure.3BB) and the slope 2 (Figure.3BC). 26 samples were tested to match the results of identification of urine cultures with current isometric illustrations. There were 18 samples that can be tested suitability and 18 samples cannot be analyzed because it does not give a typical picture. The 18 samples had bacterial counts <105 CFU/mL. The slope of 1 was 66.7% according to the culture of urine, whereas on the slope 2 there was 93.3% according to the culture of the urine (Table 1). The results of the examination of leukocyte counts with 40/µL cutoff points were 28 (32.56%) of the samples, and 58 (67.44%) of

the samples were not-UTI (Table 2). The sensitivity of leukocyte examination with cutting point 40/μL to the culture of urine was 65.4%, with specificity 81.7%. The positive predictive value and negative predictive value was 60.7% and 84.5% respectively. The diagnostic value of urine leukocyte examination using flow cytometry (Table 3).

The ROC curve formed for examination of urine leukocyte counts using flow cytometry at the cutting point ≥40/μL against urine culture results ≥ 5x104 CFU/mL (Figure.5A). The under the curve (AUC) area was 0.793 (medium). p <0.05. (95% CI 0.69 - 0.89). When using the cutoff point 30/μL then obtained the sensitivity of 69.2% and specificity 71.7%. The results of the examination of the number of bacteria with 125/μL intake point were 21 samples (24. 4%) and 65 samples (75.6%) samples instead of UTI (Table 2). The sensitivity of examination of urine bacteria with the 125/μL cutting point for urine culture was 69.2%, with specificity 95.0%. Positive predictive values and negative predictive values were 85.7% and 87.7% (Table.3) respectively. The ROC curve formed for examination of the number of urine bacteria using flow cytometry at the point of ≥ 125/μL against the urine culture result ≥ 5x104 CFU/mL (Figure.5B). The under the curve (AUC) area was 0.946 (very good) .p <0.05. (95% CI 0.902-0.989). AUC obtained on bacterial examination is wider than in urine leukocyte examination.

Discussion

Based on the results obtained, there was the growth of 3 kinds of colonies of germs or more without dominant species that considered as contaminants. If there were 3 or more different organisms in urine culture then a strong allegation of shelter errors and handling of urine samples. But it can also be found in patients who use permanent urinary catheters [12].

There is a growth of fungi in urine culture results, these results are consistent with earlier studies, in which UTI may also be caused by pathogens (fungi, parasites, and viruses) colonizing the urinary tract. Mushroom (Candida spp) is one of the causes of UTI [13–15]. The presence of fungi in urine culture is an indicator of pyelonephritis that derived from hematogenous spread (descending path). Haematogenous spread is usually as a result of bacteremia or in patients with immune system disorders [6].

The result of urine culture obtained significant growth. These results were different from previous studies, the difference may be due to the screening of patients suspected of strict UTI. When more rigorous screening was performed then the possibility of a positive urine culture percentage will be even more. However, it is also known that the main problem of urine culture examination lies in the collection of samples, the length of urine in the bladder, the density of the urine, if the low urine weight may be due to increased urinary frequency, the number of colonies obtained is also low. Sampling and transportation of samples, culture techniques and interpretation of results also greatly influence urine culture test results [12]

The incidence of UTI depends on age and sex [2,5]. In this study, girls with UTI were more likely to be in the age group 0-1 years and over 5 years, while the 1-5-year-olds were more likely to suffer from boys. Girls are more at risk of developing UTI by the first year of life than boys because of different anatomical features. Moisture of the periurethral and vaginal areas stimulates the growth of uropathogenic. A shorter urethral size increases the chances of ascending infection to the urinary tract. Besides, women are more likely to receive UTI because the uropathogenic, which is part of the normal flora of the stool, can colonize in the perianal which can then rise to the vaginal introitus. The spread of uropathogenic into the periurethral, urethra and bladder can be inhibited by the normal vaginal flora (Lactobacillus sp) causing the acidic vaginal pH. If the vaginal flora is also impaired it can cause uropathogenic to stick to the urinary tract and multiply and the incidence rate of UTI will increase in estrogen deficiency [6,15].

The most common pathogenic bacteria causing UTI were Escherichia coli (E. coli). E. coli was associated with childhood UTI in developing countries. Bacteria can survive and replicate because they have virulence factors such as expression of fimbriae, synthesis of aerobactin and enterobactin (iron-binding protein), which is very high affinity to iron, useful for bacterial replication, producing hemolysin, glycocalyx-mediated adherence, somatic Ag expression, producing urease, may move and be resistant to serum bactericidal activity [15]. There was a significant number of urine bacteria between group UTIs rather than UTIs. p <0.05, so the number of bacteria with current cytometric examination can be used for screening examination of suspected UTI patients, thus reducing the number of urine culture requests. However, it is known that direct bacterial

examination cannot distinguish between uropathogenic or contaminants [15]

There were two images of the slope of the scattergram bacteria (scattergram B1), which a slope of 1 that formed from densely dispersed points and extends along a diagonal line. This describes was suspected of the bacteria in the form of coccus. Tilt 2 was the slope formed from the points collected/concentrated in the narrow zone. This picture of the possibility of urine bacteria in the form of stems [16]. The compatibility between the slope 2 and the shape of the stem bacteria was 93.3%, while for slope 1 only 66.7%. The same result was obtained in the previous research which got 2 slope suitability of 100% while the slope of 1% was 75% from 47 samples under study [16]. The formation of a typical slope image is caused by the working principle of bacterial examination using a red (semiconductor) laser that concerns the bacterial distribution of B_FSC (describes particle size) and B_FLH (describes the intensity of the nucleic acid color of the particles) [14]

The result of the analysis shows that there was a significant correlation between urinary leukocyte count and the number of colonies, but the relationship was weak, it indicated by the correlation coefficient (Spearman) only 0.376. The flow of urine can usually clear the urinary tract of pathogens. Urine itself also has specific antimicrobial properties, including low urine pH, high urea content, high organic acid content, polymorphonuclear cells, and Tamm-Horsfall glycoprotein, which can inhibit bacterial adherence in the bladder mucosal wall [6,15]. The relationship between urine bacteria and the number of colonies was quite strong, this indicated by the value of the correlation coefficient (Spearman) that quite large by 0.729. Urine bacteria found in current cytometric examination was associated with positive urine culture results but direct bacterial examination cannot distinguish between uropathogenic or contaminants [15].

Conclusion

Based on the results of research and discussion can be concluded that there was a weak relationship between urine leukocytes and the number of bacterial colonies, but a strong relationship between urine bacteria with the number of bacterial colonies.

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