INDONESIAN JOURNAL OF
CLINICAL PATHOLOGY AND MEDICAL LABORATORY
Majalah Patologi Klinik Indonesia dan Laboratorium Medik

PAGE 269
2022 July; 28(3): 269-273
p-ISSN 0854-4263 e-ISSN 2477-4685
Available at www.indonesianjournalofclinicalpathology.org

Cut-off Values of Bacteriuria and Leukocyturia to Diagnose Urinary Tract Infection in Patients Aged 0-12 Months

Rahmania Chandra Savitri[1], Dian Ariningrum[2], Yusuf Ari Mashuri[3]

[1] Faculty of Medicine, Sebelas Maret University, Surakarta, Indonesia. E-mail: rahmaniachandra@yahoo.com
[2] Department of Clinical Pathology, Faculty of Medicine, Sebelas Maret University/Dr. Moewardi Hospital, Surakarta, Indonesia
[3] Laboratory of Parasitology, Faculty of Medicine, Sebelas Maret University, Surakarta, Indonesia

ABSTRACT

Urinary Tract Infections (UTIs) are among the most common bacterial infections in the community, including among infants and children. Urine culture is the gold standard test for UTI detection, but it is expensive, impractical, and tedious. At present, automated urinalysis methods have been used by many for UTI screening. This study aimed to determine the cut-off values of leukocyturia and bacteriuria using the flow cytometry method using the automated analyzer Sysmex UX-2000 to screen UTI in patients aged 0-12 months (infants). This diagnostic cross-sectional study was conducted at the Dr. Moewardi Hospital in Surakarta in August-October 2019. Thirty-four patients underwent urine culture examination and automatic urine analyzer using Sysmex UX-2000. Cut-off values of leukocyturia and bacteriuria were determined from the ROC curve, followed by the determination of sensitivity, specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV), accuracy, and likelihood ratio. The cut-off value for leukocyturia in infant patients aged 0-12 months was 46.2 cells/μL with a sensitivity value of 88%, specificity 68%, PPV 50%, NPV 94%, accuracy 73%, and likelihood ratio 2.77. The cut-off value of bacteriuria in infant patients aged 0-12 months was 247.6 cells/μL with a sensitivity value of 66%, specificity 64%, PPV 40%, NPV 84%, accuracy 64%, and likelihood ratio 1.85. Cut-off values of leukocyturia and bacteriuria using automatic urine analyzer Sysmex UX-2000 with NPV >80% can be used for UTI screening in infant patients aged 0-12 months.

Keywords: Urinary tract infections, leukocyturia, bacteriuria, cut-off value, Sysmex UX-2000

INTRODUCTION

Urinary Tract Infection (UTI) is the presence of pathogenic bacteria in the urinary tract.[1] Urinary tract infection is an infectious disease that often occurs in infants and children. The incidence of first-time UTI is highest during the first year of life, specifically 2.7% for boys and 0.7% for girls.[2] Risk factors for UTI in infants are mostly caused by abnormalities of the anatomical and functional urinary tract, and accompanying infections can increase the risk of UTI.[3] In most cases (60-80%), UTI is caused by *Escherichia coli*.[2]

The gold standard for the diagnosis of UTI is urine culture. Each bacteria will form a colony that is counted and adjusted per milliliter of urine (colony forming unit (CFU/mL).[4] Urinary tract infection diagnosis can be established if the number of bacteria >105 CFU/mL from a clean-catch urine specimen, >103 CFU/mL from the catheter urine specimen and any amount if the urine specimen is taken using suprapubic aspiration technique.[5]

Although urine culture is the gold standard for UTI detection, this examination tends to be expensive, impractical, and tedious; not all health services have facilities for culture examinations. Therefore, a urinalysis method is used to detect bacteria and inflammatory reactions as a presumptive diagnosis of UTI.[6] Currently, the automated urinalysis method has been widely used in hospitals and laboratories. This system uses flow-imaging analysis technology to classify the particles contained in urine quickly.[7]

This study aims to determine the cut-off value of bacteriuria and leukocyturia using an automated urine analyzer to diagnose UTIs in patients aged 0-12 months in Dr. Moewardi Hospital, Surakarta. Currently, there are no studies on the cut-off values of leukocytes or bacteria in the urine to diagnose UTIs in neonates and infants.

METHODS

The research was a diagnostic study with a cross-sectional design conducted at the Clinical

Pathology Laboratory Dr. Moewardi Hospital, Surakarta, during August 2019-October 2019, using data from October 2018-October 2019. Sampling was done by total selection. The samples used were patients aged 0-12 months who had urine culture examinations and automated urinalysis. Urine culture was the gold standard for diagnosing UTIs. The bacteria in urine would form a colony that was counted and adjusted per milliliter of urine (colony forming unit (CFU/mL).) There were two methods to collect urine samples, the mid-stream technique and the catheter technique. UTI diagnosis was established if the number of bacteria was>105 CFU/mL from a mid-stream urine specimen and >103 CFU/mL from the catheter urine specimen.

The data taken were characteristics of subjects consisting of gender, risk factors (anatomical abnormalities of the urinary tract, abnormalities of urinary tract function, inflammation, and infection), methods to take urine samples, and the results of urine leukocyte (WBC) and urine bacteria (BACT).

Characteristics of subjects were tested using the Chi-Square test, while WBC and BACT data were tested using the Mann-Whitney test. The amount of WBC and BACT results were then analyzed using the ROC curve. The analysis results displayed sensitivity, specificity, accuracy, and cut-off values for the diagnosis of UTI. The best cut-off value was chosen based on the highest accuracy value. Then a diagnostic test of the cut-off value was carried out to obtain sensitivity, specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV), accuracy, and likelihood ratio.

This study has received ethical approval from the Health Research Ethics Commission of Dr. Moewardi Hospital with number 888/VII/HREC/2019.

RESULTS AND DISCUSSIONS

Thirty-four subjects met the study criteria, consisting of 9 patients (26.5%) with positive urine culture results and 25 patients (73.5%) with negative urine culture results. The youngest patient in this study was 0 months (8 days), and the eldest was 11 months. Based on Table 1, the number of female patients was 20 (58.8%), while there were 14(41.2%) male patients.

There were four patients (11.8%) who had ≤ 1 risk factor, with one (25%) having a positive culture and three patients (75%) with a negative culture. Thirty patients (88.2%) had >1 risk factor, eight patients (26.7%) with positive culture results, and 22 patients (73.3%) with negative culture results. There were 6 (20.7%) patients underwent a urine culture test with the mid-stream technique with positive culture results and 23 (79.3%) with negative culture results. In comparison, the catheter collection technique involved three patients (60%) with positive culture results and two (40%) with negative culture results.

Table 2 shows the types of bacteria that grew in urine culture with positive results in 9 patients (26.5%). The most common bacteria was *Klebsiella pneumoniae* in 5 patients (55.6%). Other bacteria found were 2 (22.2%) *Escherichia coli*, one (11.1%) *Acinetobacter baumannii* and one (11.1%) *Stenotrophomonas maltophilia*.

Table 2. Types of bacteria on positive urine culture results

Bacteria	n	%
Klebsiella pneumoniae	5	55.6
Escherichia coli	2	22.2
Acinetobacter baumannii	1	11.1
Stenotrophomonas maltophilia	1	11.1
Total	9	100.0

Table 1. Characteristic of subjects

Characteristic	Urine Culture (+)		Urine Culture (-)		Total		p-value
	n	%	n	%	n	%	
Gender							
Female	6	30	14	70	20	58.8	.704
Male	3	21.4	11	78.6	14	41.2	
Risk factors							1.000
≤ 1 Risk factor	1	25	3	75	4	11.8	
> 1 Risk factor	8	26.7	22	73.3	30	88.2	
Methods of urine collection							
Mid-stream	6	20.7	23	79.3	29	85.3	.102
Catheter	3	60	2	40	5	14.7	

The characteristics data obtained was then tested using the Chi-Square test to compare the nominal-scale variables. Chi-Square results in this study can be seen in Table 1. Obtained p-values was > 0.05 in all variables tested. This result shows no significant difference in the proportion of gender, risk factors, and urine collection methods based on urine culture results.

Furthermore, an unpaired non-parametric comparative Mann-Whitney test was used to test ratio-scale variables: the number of urine leukocytes (WBC) and urine bacteria (BACT). Based on Table 3, p <0.05 was obtained for WBC, meaning there was a significant difference in the median urine leukocytes based on culture results. In contrast, BACT obtained p > 0.05, which shows no considerable proportion difference in the median of urine bacteria based on urine culture results.

The results of the number of WBC and BACT using Sysmex UX-2000 were then analyzed using the Receiver Operating Characteristic (ROC) curve to find the best sensitivity for UTI screening and the best specificity for UTI diagnosis and to obtain WBC and BACT cut-off values. The ROC curve can be seen in Figure 1.

After being analyzed using the ROC curve, the best cut-off value for urine leukocytes (WBC) was 46.2 cells/µL, and the cut-off value for urine bacteria (BACT) was 247.6 cells/µL. Then a diagnostic test was performed to determine the sensitivity, specificity, PPV, NPV, accuracy, and likelihood ratio. Based on Table 4, the cut-off value for WBC was 46.2 cells/µL with a sensitivity value of 88%, specificity of 68%, PPV of 50%, NPV 94%, accuracy of 73%, and a likelihood ratio value of 2.77. For BACT, a cut-off value of 247.6 cells/µL was obtained with a sensitivity value of 66%, specificity of 64%, PPV of 40%, NPV of 84%, accuracy of 64%, and a likelihood ratio value of 1.85.

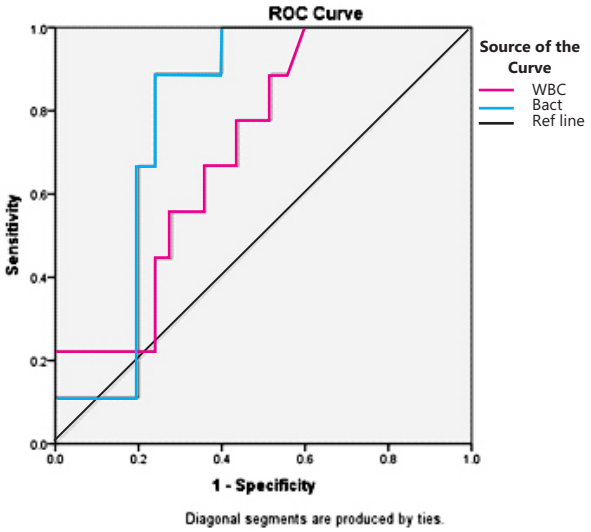

Figure 1. ROC curve for WBC and BACT

The number of subjects who met the inclusion and exclusion criteria was 34. The youngest patient in this study was 0 months old (8 days), and the oldest patient was 11 months old. Neonates and infants are at higher risk of developing a UTI due to an immature immune system.[8] According to a study, the incidence of UTIs in febrile infants, is between 10.7% -15.4%, and the incidence of UTIs within the first 3 days of life is reported to be rare (0% -1%).[9] Based on gender, 58.8% of female patients and 41.2% of male patients were obtained. These results were not much different from one study involving 84 research subjects with 50% female patients.[10] However, these results differ from the theory that UTIs were more common in boys (3.7%) in the first year of life than in girls (2%).[5]

Table 3. Mann Whitney test results of WBC and BACT

Parameter	Urine Culture (+) Median (range)	Urine Culture (-) Median (range)	p-value
WBC (cell/µL)	88.5 (27.9–182.5)	19.7 (0.5–181.8)	.011
BACT (cell/µL)	400.4 (39.5–44914.4)	51.2 (0–6283.7)	.073

Table 4. Result of sensitivity, specificity, PPV, NPV, accuracy, and likelihood ratio for WBC and BACT

	WBC 46.2 cells/µL	BACK 247.6 cells/µL
Sensitivity (95% C.I)	88% (50%-99%)	66% (30%-90%)
Specificity (95% C.I)	68% (46%-84%)	64% (42%-81%)
PPV (95% C.I)	50% (25%-74%)	40% (17%-67%)
NPV (95% C.I)	94% (70%-99%)	84% (59%-95%)
Accuracy	73%	64%
Likelihood ratio (95% C.I)	2.77 (1.49-5.14)	1.85 (0.92-3.72)

Positive urine culture results were obtained in 26.5% of patients, and negative culture results were obtained in 73.5%. The most common bacteria found in positive culture results was *Klebsiella pneumoniae* (55.6%) then, followed by *Escherichia coli* (22.2%). Although *Klebsiella pneumoniae* is one of the bacteria that cause UTI, this is not following several theories and studies that suggest that the most common cause of UTI is *Escherichia coli*.[2,9,11,12] However, some ideas support the results of this study, namely that in children and infants, *Klebsiella sp.* more often causes UTI.[4]

The existence of risk factors such as abnormalities of urinary tract anatomy, abnormalities of urinary tract function, inflammation, and infection are risk factors for UTIs. Urinary tract anatomical abnormalities found in patients in this study included cryptorchidism, ureteral atresia, ureteric stenosis, and phimosis. Urinary tract function abnormalities found were nephrosis syndrome, kidney failure, hydronephrosis, and hydroureter. Inflammation was found in almost all patients because most patients experience infections, including UTI, pneumonia, peritonitis, and sepsis.

Research shows that 21.9% of children with a UTI had an identified congenital kidney and urinary tract anomaly, most commonly hydronephrosis (12.9%) and vesicoureteral reflux (10.2%).[13] Other studies have also suggested something similar where prenatal hydronephrosis is one of the most common anomalies detected on prenatal ultrasonography. Patients with prenatal hydronephrosis and ureteral dilation are at increased risk of UTI. Research shows that patients with ureters 7 mm or greater had nearly three times the risk of UTI adjusting for hydronephrosis grade.[14]

In another study, it was found that 8.33% of patients had anatomic abnormalities in the form of phimosis.[15] Phimosis caused disruption in the flow of urine by difficulty urinating, reduced urine flow, bulged the tip of the prepuce of the penis during micturition, and causing urinary retention. Unclean local hygiene caused UTIs. Bacteria originating from the normal flora of the prepuce, epithelial surface, and distal urethra can attach to the inner layer of the prepuce and the uroepithelial cells.[15]

Mann-Whitney test results provided significant results for the variable number of urine leukocytes (WBC) and insignificant results for the number of urine bacteria (BACT). Based on culture results, there was a substantial difference in the WBC median and no difference in the BACT median. There were high values of leukocytes and bacteria in negative culture results. False-positive results could be caused by several things, including the contamination of urine samples, delay in the examination of urine samples resulting in bacterial colonization, and the presence of other inflammatory processes that are not accompanied by infection. In addition, the Sysmex UX-2000 machine counts all live and dead bacteria, unlike cultures that only grow living bacteria.

This study's cut-off value for WBC was 46.2 cells/µL with a sensitivity value of 88%, specificity of 68%, PPV of 50%, NPV 94%, and accuracy of 73%. Other studies have a cut-off value that does not differ significantly, 52.8 cells/µL with a sensitivity value of 82.3%, specificity of 76.3%, PPV 85%, NPV 72.5%, and an accuracy of 80%.[16] A study at the same hospital as this study but with different populations gave higher WBC cut-off values of 125.85 cells/µL with a sensitivity of 67.6%, specificity of 57.2%, PPV of 21.1%, and NPV of 91.3%.[12]

The BACT variable in this study produced a cut-off value of 247.6 cells/µL with a sensitivity value of 66%, specificity of 64%, PPV 40%, NPV 84%, and an accuracy of 64%.

Variation of the cut-off values in the number of leukocyturia and bacteriuria in diagnosing UTI can be caused by several things, including the differences in sample characteristics included in the inclusion criteria, differences in population from each study, environmental factors that influence the prevalence of UTI in each region and differences in definitions in the number of urine cultures as positive UTI used in the study.[16]

This study has a high NPV of 94% for WBC and 84% for BACT. The high NPV of leukocyturia and bacteriuria in this study showed the ability of the two parameters to predict the results of negative urine culture by 94% and 84% to rule out a UTI diagnosis. So it can be concluded that the cut-off of 46.2 cells/µL for leukocyturia and 247.6 cells/µL for bacteriuria can be used for UTI screening in patients aged 0-12 months. Another advantage of this study is that no other studies examine the urinalysis cut-off in these populations.

The limitation of this study is the small number of samples with an unbalanced proportion of positive and negative culture results. This finding was caused by not all patients having urine cultures and urinalysis tests. Furthermore, not all patients had a urinalysis examination using the Sysmex UX-2000 machine. There was also unaccepted and incomplete data excluded, causing a reduction in the total sample size. In addition, several patients had culture examinations and urinalysis on different days. This fact can cause a change in the urine profile, so the results of the tests are less valid. Another limitation of

this study is it was retrospective, rendering researchers unable to control the factors that can affect the results of urine culture or automated urinalysis, for example, how to take samples, time of examination, and administration of antibiotics. Patients examined at Dr. Moewardi Hospital are most likely already given antibiotics, causing a low number of bacteria and leukocytes in several examinations.

CONCLUSIONS AND SUGGESTIONS

The cut-off value of WBC of 46.2 cells/μL with 88% sensitivity, 68% specificity, PPV 50%, NPV 94%, 73% accuracy, the likelihood ratio of 2.77, and a cut-off value of BACT of 247.6 cells/μL with a sensitivity of 66%, specificity 64%, PPV 40%, NPV 84%, an accuracy of 64% and a likelihood ratio of 1.85 can be used for UTI screening in patients aged 0-12 months. However, further research is needed with a larger sample size and prospective research so that the results are more accurate.

REFERENCES

1. Gomella TL, Cunningham MD, Eyal FG (eds). Neonatology: Management, procedures, on-call problems, diseases, and drugs. 8th Ed., New York, McGraw-Hill Education, 2020; 932-933.
2. Riccabona M (ed). Pediatric urogenital radiology. 3rd Ed., Switzerland, Springer International Publishing, 2018; 219-224.
3. Align B, Abebe B, Shibeshi A, *et al*. Bacterial isolates and their antimicrobial susceptibility patterns among pediatric patients with urinary tract infections. Turk J Urol, 2018; 44 (1): 62-69.
4. McAninch JW, Lue TF (eds). Smith & Tanagho's general urology. 19th Ed., New York, McGraw-Hill, 2020; 197-201.
5. Tekgül S, Dogan HS, Hoebeke P, Kocvara R, Nijman JM, Radmayr C, Stein R. Guidelines on paediatric urology. Arnhem, European Association of Urology, 2017; 29-45.
6. Oyaert M, Delanghe J. Progress in automated urinalysis. Ann Lab Med, 2019; 39(1): 15-22.
7. Tej KM, Nader S, Caleb PN. Contemporary management of urinary tract infection in children. Pediatrics, 2021; 147 (2): 1-12.
8. Korbel L, Howell M, Spencer JD. The clinical diagnosis and management of urinary tract infections in children and adolescents. Paediatr Int Child Health, 2017; 37(4): 273-279.
9. Arshad M, Seed PC. Urinary tract infection in the infants. Clin Perinatol, 2017; 42(1): 1-16.
10. Taufik MRI, Ariningrum D, Mashuri YA. Cut-off values of bacteriuria and leukocyturia for the diagnosis of urinary tract infections in pediatric patients. Indonesian Journal of Clinical Pathology and Medical Laboratory, 2020; 27(1): 51-54.
11. Pardede SO. Infeksi pada ginjal dan saluran kemih anak: Manifestasi klinis dan tata laksana. Sari Pediatri, 2018; 19(6): 364-374.
12. Patricia T, Ariningrum D, Prijambodo J. Penetapan Cut-off leukosituria dan bakteriuria menggunakan urine analyzer Sysmex UX-2000 untuk skrining infeksi saluran kemih. Surakarta, Program Studi Patologi Klinik Fakultas Kedokteran Universitas Sebelas Maret Surakarta, 2019; 18-19.
13. Chang JW, Liu CS, Tsai HL. Vesicoureteral reflux in children with urinary tract infections in the inpatient setting in Taiwan. Clin Epidemiol, 2022; 14: 299-307.
14. Holzman SA, Braga LH, Zee RS, Herndon CDA, Dao CAD, *et al*. Risk of urinary tract infection in patients with hydroureter: An analysis from the society of fetal urology prenatal hydronephrosis registry. Journal of Pediatric Urology, 2021; 17(6): 775-781.
15. Tusino A, Widyaningsih N. Karakteristik infeksi saluran kemih pada anak usia 0-12 tahun di RS X Kebumen Jawa Tengah. Biomedika, 2017; 9 (2): 39-46.
16. Pratistha FSM, Sudhana IW, Adnyana IWL. Diagnosis cepat infeksi saluran kemih dengan menghitung jumlah leukosituria pada urinalisis metode flow cytometry Sysmex UX-2000 dengan baku emas kultur urin di RSUP Sanglah Denpasar. E-Jurnal Medika Udayana, 2018; 7(5): 211-216.