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FULL PAPER

Performance of Sysmex UF-5000 for candiduria screening

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SUMMARY

In this study, we tested the performance of the Sysmex UF-5000 system to detect yeast-like cell (YLC) counting to screen for candiduria. Urine samples were screened for leukocyte and yeast amount by flow cytometry and results were compared with fungal culture results. A total of 56,749 urine samples were enrolled in this study. Urine culture and urinalysis of YLC data were used to evaluate the performance of YLC in diagnosing candiduria. Different cut-off values (YLC. 5, 10, 20, 50, 100/µl) were evaluated. Youden index was used to determine the ideal cut-off value, and the highest was 0.95 with 5 YLC/µl. When the cut-off value for YLC is 5 cells/µl, 95.15% of the samples were "negative" with flow cytometry and culture (NPV:100%). In conclusion, detection of YLC by flow cytometer (Sysmex UF-5000) can be a rapid alternative method to exclude candiduria prior to urine culture.

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INTRODUCTION

Candiduria, defined as the presence of *Candida* spp. in urine, is increasingly becoming an important cause of nosocomial urinary tract infection (Lundstorm 2001). *Candida* spp. frequently cause urinary tract infections (UTI) in patients with co-morbidities and in patients who have underlying predisposing disorders, renal and collecting duct anomalies (Kauffman 2000). Antibiotics, corticosteroids, immunosuppressive agents, and urinary catheters, all of which are used extensively in clinical practice, are possible risk factors. *Candida* spp. account for 20% of the UTIs in ICUs. They are the second leading pathogen causing UTI in ICUs after *Escherichia coli* (Platt, 1986; Padawer, 2015). In patients with UTI, an empirical treatment is generally initiated based on the frequently encountered bacteria and yeasts are usually ignored. In addition, in mixed infections caused by bacteria and yeasts, bacteria become dominant because their half-lives are shorter, as a result of which pathogen yeasts can be overlooked. Therefore, inadequate treatment may be administered to these patients. Urine culture is the primary method to identify candiduria in clinical laboratories. However, urine fungal culture is labor-intensive and time-consuming; therefore, a simple method for candiduria identification is urgently needed. Thus, rapid and accurate screening of urine samples for candida infection will be able to eliminate these downsides. Flow cytometry has been considered reliable and useful for detection of bacteria in urine. However, there are limited studies of candida infections (Andreu, 2011; Jimenez Guerra, 2017; Huls, 1992). Being one of these flow cytometry methods, the latest third-generation urine flow cytometer UF-5000 (Sysmex Corporation, Kobe, Japan) counts yeast-like cells (YLC) besides erythrocytes, leucocytes, epithelial cells, granular cylinders, crystals, and bacteria by using fluorescent flow cytometry based on diode laser technology. In this study, we screened urine samples for the presence of YLC by Sysmex UF-5000 before culturing and evaluated the performance of the system in comparison with the fungal culture results.

Key words:
Candiduria, Urine culture, Flow cytometry, UF-5000, Urinalysis.

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METHODS

Urine samples submitted to the clinical microbiology laboratory of our hospital for culture from February 2018 to November 2020 were enrolled in this study. The samples were collected as midstream urine from adults. For babies under 1 year, sterile urine bags were applied to collect urine (Procop, 2017). For culture; samples were inoculated on blood agar and on chromogenic media (chrom ID CPS3, bioMerieux, France) by calibrated loop (10-

µl) and incubated at 35-37°C for 18-24 h. In case of no growth, incubation time was prolonged to 48 hrs. (Tille 2014). Identification of species was performed by MaldiTof-MS (Biomerieux, France). Samples were simultaneously analyzed for yeast (YLC) and leucocyte counts by flow cytometry (Sysmex UF-5000) YLC counts of the UF-5000 were compared with the urine culture results. RBC/YLC abnormal classification is displayed when RBC ≥20/µL and large numbers of YLC with low forward scattered light and fluorescence intensity are present, which results in overlap of RBC and YLC clusters on the scattergram, making them difficult to differentiate. In this study, we eliminated the flagged samples that appear in the "RBC/YLC Abnormal Classification"; therefore, the accuracy of the yeast cell is >80% and R^2:0.9972 which are declared by Sysmex in UF-5000. For statistical analysis, SPSS 21 was used. Sensitivity, specificity, positive predictive value, and negative predictive value were calculated by different cut-off values. The ideal value was determined by Youden index calculation.

RESULTS

In total, 56,749 urine samples were evaluated in our study. Of these 45.25% (25,681) were from children aged 0-17years, 54.75% were from adults [14,546 male (25.63%), 16, 52 female (29.11%)]. Culture was positive in 755 of 56,749 urine samples. Of these pathogens, 24.11% (182) were yeasts. The dominant species among the yeasts was *Candida albicans* with a rate of 43.41% (79/182). Other species were *C.glabrata* 28% (51), *C.tropicalis* 10.9% (20), *C.kefyr* 6% (11), *C.parapsilosis* 4.3% (8), *C.krusei* 2.7% (5), *C.lusitaniae* 1% (2), *C.orthopsilosis* 0.5% (1) and *T.asahii* 2.7% (5). All these 182 positive samples were also detected as positive with flow cytometric method. (sensitivity: 100%). Different cut-off values were evaluated for YLC. When the cut-off value for YLC is 5 cells/µl, 95% of the samples were "negative" with flow cytometry and the reference method (Negative predictive value: 100%). The samples with no yeast growth were also negative with the flow cytometry method (specificity: 95.46%). Fungal growth was detected in 35 samples by culture and all these samples were detected as positive by flow cytometric method (sensitivity: 100%). When the cut-off value for yeast-like cell (YLC) count was determined as 5 cells/µl, 24,920 samples were "negative" with flow cytometry. These samples showed no growth with the reference method (NPV: 100%). When the samples of patients aged 18-65 years were evaluated, 13,526 samples of female patients were analyzed and yeast growth was found in 55 samples. When the cut-off value was determined as 10 cells/µl, both NPV and sensitivity reached 100%, while specificity was 92.17%. In male patients, 9,818 samples were analyzed and 28 were positive for yeast NPV. Sensitivity reached 100% when cut-off value was 20 cells/µl. In this cut-off value, specificity was 97.54%. In our study, 7,724 samples were collected from elderly patients over the age of 65. Fungal growth was detected in 64 of these samples. NPV and sensitivity of flow cytometry were 100% in the cut-off value of 10 cells/µl, whereas specificity was 93.07%. Sensitivity, specificity, positive predictive values, negative predictive values and Youden index were calculated for different cut-off

Table 1 - *Sensitivity, specificity, positive predictive values (PPV), negative predictive values (NPV) and Youden index results for different threshold values.*

Cut off value (YLC/µl)	*Sensitivity %*	*Specificity %*	*(PPV) %*	*(NPV) %*	*Youden index*
5	100	95	66	100	95
10	89	95	5,9	99	84
20	69	95	5	99	65
50	51	97	6	99	49
100	40	98	7,9	99	38

Table 2 - *Comparison of the number of culture colonies with flow cytometric cell counts.*

		Number of culture colonies (cfu/ml)				
		10^3	*10^4*	*10^5*	*10^6*	*10^7*
Flow cytometry (cells/ml)	10^3	16	2	2	0	0
	10^4	34	19	22	3	0
	10^5	1	7	35	11	3
	10^6	2	1	4	6	0
	10^7	0	0	1	2	1

values (*Table 1*). As cut-off values increased, sensitivity regressed from 100% to 40.11%, and specificity increased from 95.46% to 98.50%. As PPV increased from 6.62% to 7.90%, NPV regressed from 100% to 99.80%. Youden index was used to determine the ideal cutoff value. The highest Youden index was found to be 0.9546 with 5 YLC/µl.

Of the 2,748 samples with YLC ≥5 YLC/µl in flow cytometric method, yeast growth and bacterial growth was detected in 182 and 2,235, respectively. No growth was detected in 331 of the samples. In 70% of samples with >=10^5 YLC/ml detected by flowcytometric methods, the same amount of growth was detected by culture. Yeast cell number was also determined to be 10^5 cells/ml with the flow cytometric method in 54.69% (35/64) of samples which were found to have a growth of 10^5 cfu/ml according to the culture method. This rate was found to be 27.27% (6/22) for 10^6 cfu/ml and 65.52% (19/29) for 10^4 cfu/ml. (*Table 2*). When the number of colonies isolated from the culture was statistically compared with the number of yeast cells counted withcytometry, the difference between them was significant ($p<0.001$). The coefficient of correlation between these data was 0.689.

DISCUSSION

Bacterial urinary tract infections are one of the most frequently encountered infections (Chan, 2016; McLellan, 2016). However, rarely reported *Candida* spp. in the etiology may lead to failure in empirical therapy and anti-biotherapy may harm the microbiota. As observed in studies with bacteria, the unnecessary use of antimicrobials can be prevented in no-growth samples by determining cut-off values for yeasts using flow cytometric methods. In our study, YLC value with a negative predictive value of 100% was reached in all age populations at 5 cells/µl. When this cut-off value is accepted, 95% of the urine samples can result as "no yeast cell" within half an hour after reaching the laboratory. In samples with YLC values ≥5 cells/µl according to flow cytometry, yeast growth was detected at a rate of 35.47% (182/513) of the samples when those with bacterial growth were excluded. In samples with YLC ≥5 cells/µl and pure or mixed bacterial growth, presence of yeasts should also be suspected. Bacteria can lead to the suppression of yeast growth by becoming dominant during the incubation process since their half-lives are much shorter than those of yeasts. Although more advanced studies are required, it may be concluded based on the available statistical data that yeasts were present in approximately 800 samples (2235x0.3547) in this group. Candiduria should be kept in mind besides antibiotic resistance,

particularly in patients without any improvement despite the administration of antibiotic therapy. Zhengxin et al. have determined sensitivity, specificity, negative predictive values and positive predictive values for different cutoffs in their study carried out with Sysmex UF-1000 (Zhengxin, 2019). The results of sensitivity, specificity and negative predictive values obtained in this study were comparable with the results in our study. On the other hand, positive predictive value was higher than in our study. When we determine a cut-off value with a high positive predictive value, the negative predictive value also decreases below 100%. In our study, the cut-off value with the highest Youden index was 5 YLC/µl. Since this is the same value with a 100% negative predictive value, it stands out as the most suitable value to be used in our results. In another study by Sysmex UF-1000i, the cut-off for YLC was accepted as 100 cells/µl (Jie, 2010). Sensitivity was 86%, specificity was 95%, positive predictive value was 91% and negative predictive value was 94% in this study. Similarly, our statistical data other than positive predictive values were also found to be rather high in our study. Considering the fact that flow cytometry method can be used for screening purposes, we aimed to determine all samples with growth and cut-off values with 100% negative predictive value. In a study carried out by Guiterrez-Fernandez *et al*., 22,132 urine samples were rescreened using Sysmex UF-1000i (Jose, 2014). Different threshold values were not compared but sensitivity, specificity, negative predictive values and positive predictive values were determined at a single threshold value (YLC≥50). Similar to the results obtained in our study, data other than positive predictive values were also found to be quite high in this study. Although the negative predictive value was 99.9%, a value below 100% means that normally a urine sample containing yeasts would be negative with flow cytometry and thus would not undergo inoculation, which is contradictory to the purpose of our study. Therefore, in this study we aimed to determine the highest threshold value with a 100% negative predictive value. More no-growth samples can be eliminated by identifying different threshold values for different age groups and recording them into the system. Previous studies have shown that screening urine samples with flow cytometry can help eliminate the negative urine samples and detect positive samples (Jiménez-Guerra, 2017; Angulo, 2020; Daldaban, 2019; Pieretti, 2010; İnigo, 2016; Martin-Gutierrez, 2015; Ilki, 2017). Direct urine identification can also reduce the use of empirical and/or inappropriate antimicrobials, resulting in cost effectiveness by rapidly responding to the clinic in no-growth samples with the use of flow cytometry method. In addition, it provides supportive information about the possibility of a yeast infection in samples with more than one microorganism and in patients with urinary tract infections not responding to antibiotic therapy.

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