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Rapid diagnosis of uncomplicated urinary tract infection with laser flow cytometry

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ABSTRACT

Objective: To evaluate the performance of Sysmex UF1000i (Sysmex, Kobe, Japan) in predicting significant bacteriuria on urine culture from urine specimens of women suspected of uncomplicated urinary tract infection (uUTI).
Materials and methods: We retrospectively reviewed urine samples from 1680 adult women with lower urinary tract symptoms suggestive of uUTI from urologic clinics for study. Urine analyses were performed with laser flow cytometry (Sysmex UF1000i). Standard urine cultures were performed. The predictive validity for significant bacteriuria ($\geq 10^5$ CFU/mL) was analyzed with logistic regression. Receiver operating characteristic (ROC) curve analysis was used to evaluate the best cut-off point for bacteria count (BACT/μL) to predict significant bacteriuria.
Results: A total of 651 specimens met the criteria for analysis. The results indicated that the BACT/μL (AUC = 0.94) generated through Sysmex UF1000i outperformed nitrite (AUC = 0.70), leukocyte esterase (AUC = 0.71), and pyuria (AUC = 0.678) in predicting significant bacteriuria. The optimal cut-off point for bacterial count was set at > 357 BACT/μL with sensitivity of 85.61%, specificity of 88.52%, positive predictive value of 91.8%, and negative predictive value of 78.73%. Excluding those specimens with < 357 BACT/μL, we may decrease negative urine culture rate by 36.9%, and miss 14.0% of positive cultures.
Conclusion: Laser flow cytometry (Sysmex UF1000i) can help us rapidly identify patients with significant bateriuria in the preanalytical phase urine culture and thus reduce unnecessary use of antibiotics.

1. Introduction

Urinary tract infection (UTI) is one of the most common bacterial infections at all levels of healthcare.[1] The uncomplicated UTI (uUTI) is defined as infection in patients with normal genitourinary tract, while the complicated UTI is infection in those with abnormal genitourinary tract, immunocompromised status, and/or multidrug resistant bacteria.[1] By the age of 32 years, 50% of women would have had at least one symptomatic UTI.[2] The diagnosis of UTI is made through clinical symptoms and a positive urine culture.[1] Urine culture remains the gold standard for diagnosing UTI, by identifying the causative pathogen with concentration and susceptibility test.[1] High percentages of negative culture results, ranging from 80–98%, have been reported.[3] Also, contamination accounted for 29–32% of positive urine cultures in symptomatic female outpatients, despite different collection methods.[4] However, 23% of symptomatic women exhibited negative urine cultures.[5] Urine chemical analysis and microscopic testing, including leukocyte esterase, nitrite, pyuria, and bacteriuria, rapidly provide information in order to make a diagnosis of uUTI. Despite exhibiting good diagnostic performance for the detection of UTI, their lack of accuracy and precision are still being debated.

A new advanced automated urine flow cytometer, Sysmex UF1000i (Sysmex, Kobe, Japan), has recently served as an alternative to the expensive, time consuming, and labor-intensive urine culture method.[6] Sysmex UF1000i is designed to analyze urine sediment at a speed of 100 samples per hour and can rapidly quantify urine particles, including bacteria, white blood cells (WBCs), red blood cells (RBCs), epithelial cells, and casts. Using a

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specific reagent and dye in a separated analytical channel, Sysmex UF1000i provides a fast, effective, and reliable screening test by detecting and quantifying bacteria before culture, which obviates the need for culture and unnecessary antibiotic use. Previous studies showed good precision and consistency between Sysmex UF1000i and urine culture and microscopic counting results.[6–12] Although large sampling numbers were included, many did not specifically note the patients' clinical status or report the incidence of contaminated urine samples. To address these issues, we performed a retrospective study to compare the performance of the bacteria count (BACT/μL) generated through laser flow cytometry (Sysmex UF1000i) with dipstick chemical analysis and pyuria in predicting bacteriuria in urine specimens from adult women with uUTI.

2. Materials and methods

2.1. Patients and samples

From October 2014 to October 2015, adult women (≥ 18 years old) visiting urological clinics with lower urinary tract symptoms suggestive of uUTI, i.e., urinary frequency, urgency, dysuria, and hematuria, were enrolled retrospectively. Samples were excluded from analysis if urine analysis and urine culture were not performed within 24 hours. Patients were also excluded if they were febrile (body temperature > 38°C), pregnant, if they had genitourinary tract anomaly, were undergoing immunosuppressive therapy, recent antibiotic use (within 1 week), chronic kidney disease under dialysis, chronic urine retention under urethral catheterization, or bladder cancer.

Midstream urine specimens were collected in sterile containers for urine analysis and culture. The urine specimens were placed in 10 mL urine sediment centrifuge tubes (SY; Shih-Yung Medical Instruments Co. Ltd, Taipei, Taiwan) and sent for chemical analysis and laser flow cytometry (Sysmex UF1000i) examination in our laboratory. Sysmex UF1000i analysis was performed within 30 minutes of collection. The study was approved by the Institutional Review Boards at the Taipei Tzu Chi Hospital, Taipei, Taiwan, with the protocol number 03-XD49-86.

2.2. Sysmex UF1000i

In our hospital, the standard urinalysis is done by Sysmex UF1000i and one chemistry analyzer. That means that ordering urine examination does not require extra expense. The results of sediment analysis were presented in units of per μL and also in per high power field (HPF). Most clinicians are not familiar with the interpretation of the results from this machine; therefore, our laboratory still provides the universal format familiar to clinicians. The Sysmex UF1000i is a fully automated fluorescence flow cytometer which utilizes a 635 nm semiconductor diode laser to quantify bacteria and sediments in two separated analytical channels without the need to centrifuge urine specimens. In the bacterial channel, the urine specimen was mixed at 42°C. A special diluent, which increases permeability of the bacterial cell wall, allowed the polymethine fluorescent dye to stain bacterial DNA. Each bacterial particle, exposed to a 635 nm laser beam, emitted various degrees of forward scattered light and side fluorescent light and could be counted to generate the BACT/μL. In the sediment channel, WBCs, RBCs, yeast-like cells (YLCs), epithelial cells, crystals, casts, spermatozoa, and mucus were analyzed. Particle/μL could be transformed to the traditional number per high power field (HPF) by multiplying specific factors. Pyuria was defined as WBC > 10/HPF.

2.3. Urine culture

A standard quantitative urine culture was performed with a 1 μL inoculation loop onto commercial chromogenic agar medium (CPS ID3, Biomerieux, Marcy I'Etoile, France). Culture plates were incubated aerobically at 35°C for 18–24 hours. Quantification (CFU/mL) was obtained by multiplying the number of colonies on an agar plate by the dilution factor. Positive cultures were defined as single bacterial growth ≥ 10^5 CFU/mL. Mixed growth was defined as growth of two or more bacteria species without a predominant organism. No subsequent species identification was performed for the category of mixed growth.

2.4. Statistical analysis

Data was expressed as mean ± standard deviation and analyzed using commercial statistical software (MedCalc Statistical Software version 16.1, MedCalc, Ostend, Belgium). The predictive validity of leukocyte esterase, nitrite, pyuria (> 10/HPF) and BACT/μL for significant bacteriuria was analyzed by using logistic regression. Receiver operating characteristic (ROC) curve analysis was used to evaluate the best cut-off point for bacteriuria with regard to significant bacteriuria. A p value < 0.05 was considered statistically significant.

3. Results

3.1. Screening of significant bacteriuria

A total of 1680 urine specimens were screened for analysis. Exclusion criteria included urine culture and urine analysis not being performed within 24 hours (n = 264), recent antibiotic use (n = 82), severe gross hematuria and cloudy urine that might cause carry over effect (n = 166), mixed growth (n = 467), growth of fungus (n = 8), lactobacillus (n = 33), and *Gardnerella vaginalis* (n = 9). Mixed growth was regarded as contamination and cannot represent the true causative pathogen in the analysis. Patients with diabetes mellitus are relatively immunocompromised and more suspected to urinary tract infection. However, in our study, we did not specify the patients' status of diabetes mellitus. Among 651 urine specimens eligible for analysis, 90 (13.8%) revealed no growth. Of the specimens with bacterial growth between 10^3 CFU/mL and 10^5 CFU/mL, 84 (54.9%) were gram negative and 69 (45.1%) were gram positive. For those with bacterial growth ≥ 10^5 CFU/mL, 369 (90.4%) were gram negative and 39 (9.6%) were gram positive.

Bacterial growth ≥ 10^5 CFU/mL was considered as a cut-off for culture-positive specimens. The identified organisms are listed in a decreasing order of prevalence: *Escherichia coli* (n = 292), *Klebsiella pneumoniae* (n = 31), *Proteus mirabilis* (n = 29), *Streptococci* (n = 23), *Enterococci* (n = 9), *Citrobacter* (n = 5), *Enterobacter* (n = 5), *Staphyloccoi* (n = 4), *Corynebacterium* (n = 3), *Pseudomonas aeruginosa* (n = 3), *Klebsiella oxytoca* (n = 1), *Klebsiella ozaenae* (n = 1), *Proteus vulgaris* (n = 1), and *Providencia rettgeri* (n = 1).

3.2. ROC curve analysis

ROC curves were used to define the optimal cut-off point of BACT/μL for predicting a positive culture (single bacterial growth ≥ 10^5 CFU/mL). At the cut-off point of > 357 BACT/μL for a positive culture, maximized sensitivity (85.89%) and specificity (88.43%), with positive predictive value of 91.80% and negative predictive value of 78.73% were obtained (Table 3). Among the 90 specimens with no growth of organisms, only four specimens exhibited BACT/μL > 357/μL in flow cytometry. When mixed growth specimens were taken into account, positive predictive value