Automated Urine Particle Analyzer UF-1000i Can Pre-Estimate the Treatment Response of Women's Uncomplicated Urinary Tract Infections to Antibiotics

Stephen Shei-Dei Yang[1], Yi-Sheng Chen[1], Chun-Chun Yang[2], Shang-Jen Chang[1]*

Purpose: To evaluate the ability of bacterial scatter diagrams generated from the automated urine particle analyzer (UF-1000i, Sysmex, Kobe, Japan) to pre-estimate the treatment efficacy of oral cefalexin in treating women with uncomplicated urinary tract infection (uUTI).

Materials and Methods: Over 3 years, women 20-80 years old with symptoms suggestive of uUTI (Urinary Tract Infection Symptoms Assessment symptom score, ,UTISA > 3) and bacteriuria (bacterial count ≥ 100/uL) were enrolled. After informed consent, patients took cephalexin 500mg 4 times/day for 7 days. The voided urine specimens were classified into rods or cocci/mixed group automatically through the built-in software of the UF1000i. Patients were followed up with UTISA on the 3rd day after treatment and returned to the clinic on the 7th day and followed for additional UTISA and urine analysis. Symptom and laboratory improvement were defined as UTISA < 4 and bacterial count < 100/uL, respectively, on the 7th day.

Results: Of 99 women (age: 49.91 ±15.32 years) eligible for analysis, 80 were classified as having urine that contained rods and 19 as cocci/mixed. Symptom improvement was observed in 62 women in the rods group and 11 women in the cocci/mixed group ($p = 0.08$). Laboratory improvement was noted in 64 women in the rods group and 10 women in the cocci/mixed group ($p = 0.01$). On day 7, treatment success with both symptom and laboratory improvement was more observed in rods than in cocci/mixed group (61.3% vs. 26.3%, $p < 0.01$).

Conclusion: The automatic urine particle analyzer can pre-estimate the treatment response of antibiotics in women with uUTI.

Keywords: uncomplicated urinary tract infection; diagnosis; laser flow cytometry; fully automated urine particle analyzer; Sysmex UF-1000i

INTRODUCTION

Urinary tract infection (UTI) due to bacteria is one of the most common infections among humans, particularly in women. More than two-thirds of women will experience at least one episode of UTI during their lifetime[1]. Although some experts suggested not using antibiotics to treat women's uUTI, empirical treatment has been frequently suggested to treat women with uncomplicated UTI (uUTI)[2]. In Taiwan, guidelines for treating UTIs suggest that nitrofurantoin, trimethoprim-sulfamethoxazole, first and second generation cephalosporin and quinolone are all reasonable choices for first line treatment[3]. Ampicillin, ampicillin/sulbactam, fluoroquinolone are regarded as alternative treatment choices. Previous studies showed that gram negative rods are the major pathogens causing urinary tract infection and have lower susceptibility rates for trimethoprim-sulfamethoxazole (49%), ampicillin (30%), ampicillin/sulbactam (34%), but high susceptibility rate (81%) for cefazolin[4]. Hence, cephalexin was chosen as the first line empirical antibiotic.

However, treatment based on clinical symptoms without urinalysis or urine culture may lead to unnecessary use of antibiotics, increasing drug resistance to antibiotics and reduce response rate of treatment. The automated urine particle analyzer, Sysmex UF-1000i (Sysmex, Kobe, Japan), has served as an alternative to the expensive, time consuming and labor-intensive urine culture[5,6]. The Sysmex UF-1000i, designated for analyzing urine sediment at a throughput of 100 samples per hour, can rapidly quantify urine particles, including bacteria, white blood cells (WBCs), red blood cells (RBCs), epithelial cells and casts[7]. Using a specific reagent and dye in a separated analytical channel, the Sysmex UF-1000i provides a fast, effective and reliable screening test by detecting and quantifying bacteria before culture. Previous studies showed that the UF-1000i could predict significant bacterial growth in urine culture based on the bacterial count[8-10]. The automated urine particle analyzer can also classify the morphology of bacteria in urine specimen into gram negative rods or cocci/mixed growth in the pre-analytical phase of urine culture with acceptable sensitivity and

[1]Division of Urology, Taipei Tzu Chi Hospital, Taipei Taiwan, and Tzu Chi University, Hualien, Taiwan.
[2]Division of General Laboratory, Taipei Tzu Chi General Hospital, New Taipei, Taiwan
*Correspondence: Division of Urology, Taipei Tzu Chi Hospital, Taipei Taiwan, 289 Jianguo Rd., Sindian District, New Taipei City, 231, Taiwan. Tel: +886-6628-9779 EXT: 5713, Fax: +886-6628-9009, E-,mail: krissygnet@gmail.com
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Table 1. Determination and comparison of demographic variables in the two groups.

	Menopause (+) n = 55	Menopause(-) n = 44	*P* value
Age (years, ±SD)	60.6 ± 7.8	34.1±10.0	P<0.001
Diabetes Mellitus, n (%)	2.4%	18.2%	*P*=0.042
Childbirth history, n (%)	35.7%	87.3%	*P*<0.001
Abdominal surgery history, n (%)	21.5%	33.6%	*P*=0.109
UTI history in the recent 1 year (%)	50.5%	50.7%	*P*=0.80
Day 0 UTISA score (mean±SD)	10.41 ± 3.73	11.09 ± 4.01	*P*=0.383

specificity[10]. Because gram negative rods have higher sensitivity to cephalexin, the identification of the morphology of bacteria (gram negative rods or cocci/mixed growth) in specimen thorough the automated urine particle analyzer may help clinicians pre-estimate the treatment response of UTI in the clinical settings. Therefore, we performed a prospective study to evaluate the treatment efficacy of cefalexin[3] in the management of uncomplicated UTI in community dwelling women and monitored the course of symptoms change. Finally, we could determine the ability of automated urine particle analyzer in pre-stimating the treatment response.

MATERIALS AND METHODS

From July 2016 to June 2019, 188 women who visited the outpatient clinic in our department with symptoms suggestive of UTI (frequency, urgency, dysuria, sense of incomplete emptying, suprapubic pain, low back pain and hematuria) were invited to join our study. The participant were asked to complete the Urinary Tract Infection Symptoms Assessment (UTISA) questionnaire[11]. The UTISA(appendix 1, 2), a valid self-admi1nistered questionnaire, has 14 items, with scores for each item ranging from 0 to 3. Among the 14 items, seven were related to severity of symptoms and seven were related to quality of life. Patients with an UTISA symptom score of > 3 were considered to have UTI and were enrolled to the study. Then, they were asked to complete a questionnaire including baseline characteristics (age, menopause status, frequency of UTI within 1 year before visit, previous abdominal surgery history, sexually active status in the past year, pregnancy status). The participants were asked to collect a mid-stream urine sample under the direction of the study nurse to lower the risk of specimen contamination. Thereafter, 10 ml of the retrieved urine sample were put into a centrifuge tube (SY, Shih-Yung medical instruments Co., Ltd, Taipei, Taiwan) for automated urine particle analysis within 30 minutes after collection of the specimen. The bacteria were then classified and counted based on their size and staining characteristics. The automated urine particle analyzer generated the scatter diagram pattern with forward scatter (B_FSC, bacteria size) and fluorescent light intensity (B_FLH, the nucleic acid content) emitted from each bacterium. For each sample with a bacteria count >100 bacteria/μL, the bacterial scatter diagram was classified as either rods or cocci/mixed by the software (Sysmex UF-1000i; TOA medical Electronics, Kobe, Japan). The remaining sample was sent for quantitative urine culture using a 1μL inoculation loop and the commercial chromogenic agar medium (CPS® ID3, Biomerieux, I′Etoile, France). The culture plates were aerobically incubated at 35℃ for 18-24h and the quantification in CFU/mL was calculated after multiplying the dilution factor and the colonies on the agar plate. In the study, urine specimens with the growth of two or more species of bacteria without a dominant one were considered to be contaminated or mixed growth.

Statistical analysis

Data were expressed as mean standard deviation and analyzed by MedCalc Statistical Software version 19.1 (MedCalc Software, Ostend, Belgium; https://www.medcalc.org; 2019). The comparisons of demographic and voiding parameters between groups were

Table 2. The sensitivity of UF1000i for specific bacteria species.

Bacterial growth of urine specimens	Number (n)	UF1000i classification
Gram (−) rods		
Escherichia coli		
≥ 10^5 cfu/mL	56	Rods=53/cocci/mixed=3
10^3-10^5cfu/mL	10	Rods=6/cocci/mixed=4
Klebsiella spp.		
≥ 10^5 cfu/mL	3	Rods=3
103-105 cfu/mL	1	Rods=1
Proteus mirabilis		
≥ 10^5 cfu/mL	5	Rods=5
103-105 cfu/mL	1	Rods=1
Citrobacter spp.		
≥10^5 cfu/mL	2	Rods=2
Gram (+) cocci		7
Streptococci spp.	3	cocci/mixed=2/Rods=1
Staphylococci spp.	3	cocci/mixed=1/Rods=2
Enterococci spp.	1	Rods=1
Group B Streptococci	1	cocci/mixed=1
Lactobacillus species	2	cocci/mixed=1/Rods=1
Two bacteria growth		
E.coli > 10^5+Streptococcus sanguinis> 10^5	1	Rods=1
Mixed growth		
≥10^5 cfu/mL	5	cocci/mixed=4/Rods=1
10^3-10^5 cfu/mL	5	cocci/mixed=3/Rods=2

performed with an independent samples t-test (continuous demographic variables), chi-squared test (nominal data), and Mann–Whitney *U*-test (ordinal data). The serially measured follow up data between groups were compared with mixed model. A *p*-value of <0.05 was considered statistically significant.

RESULTS

There were 10 women who quited after enrollment without follow up data, 2 had elevated post-void residual urine, 4 without first urinalysis, and 23 women noncompliant to medication were excluded for analysis. We also excluded 40 (27.39%) women with a bacterial count <100/μL for analysis. The baseline characteristics of the included patients (n=99) are shown in Table 1. None of the participants were pregnant at the time. Mean age and UTISA symptom scores on day 0 of the analyzed participants were 49.1 ± 15.9 years and 10.8 ± 4.0, respectively. Of the 99 participants, 44% reported to be in menopause. The mean age between rods and cocci/mixed growth group were not comparable (49.6 ± 15.9 vs. 46.5 ±16.2, $p = 0.45$). The results of the bacterial growth in urine culture and the agreement between the UF-1000i classification (rods or cocci/mixed growth) and the results of the urine culture is listed in Table 2. The analyzed specimens included 10 Gram-positive bacteria (3 Streptococci spp., 3 Staphylococci spp., 1 Enterococci spp., 1 Group B streptococci, and 2 Latobacillus species), and 11 specimens with two bacteria species or more that were regarded as mixed growth. For the Gram-negative bacteriae, there were 66 Escherichia coli, 4 Klebsiella spp, 6 Proteus mirabilis and 2 Citrobacter spp. (**Table 2**) The drug resistance to specific antibiotics of gram negative bacteria were listed in the supplement.

The mean UTISA symptom score improved from 10.8 ± 4.0 on day 0 to 2.4 ± 3.4 on day 7 ($p < 0.01$). Of the 99 women, 80 (75.5%) had urine with rods and 19 (17.9%) had urine with cocci/mixed. Symptom improvement (UTISA<4 om day 7) was observed in 62 (77.5%) and 11 (57.89%) women with rods and cocci/mixed, respectively ($p = 0.08$). The comparisons of UTISA over variable days of follow up between cocci/mixed and rods groups are shown in **Figure 1**. The symptom improvement (UTISA) in the rods group was not significantly better than the cocci/mixed group. ($p = 0.08$) Laboratory improvement was noted in 64 (80.0%) and 10 (52.63%) women with rods and cooci/mixed, respectively ($p = 0.01$). On day 7, treatment success with both symptom and laboratory improvement was observed in 49/80 (61.3%) and 5/19 (26.3%) of rods and cocci/mixed group, respectively. ($p < 0.01$).

DISCUSSION

This is the first prospective clinical study to evaluate the clinical usefulness of the automated urine particle analyzer (UF-1000i, Sysmex, Kobe, Japan) in predicting treatment response of women's uUTI to antibiotics. The symptom and laboratory improvement rate on day 7 among all included patients were 73.7% and 74.7%, respectively. Through the application of the scatter diagram generated from the UF-1000i, we may predict the treatment success of antibiotics in managing uUTI in women. Women in the cocci/mixed group had significantly lower laboratory (52.6% vs. 80.0%, $p = 0.01$) and overall treatment success rate (26.3% vs. 61.3%, $p < 0.01$). The symptom improvement rate was higher, but not statistically significant, in the rods group (77.5% vs. 57.89%, $p = 0.08$). The diagnosis of uUTI is based on clinical symptoms and bacteriuria. In the clinic, we routinely checked urine analysis for patients with uUTI for bacteriuria. The scatter diagram generated through the UF-1000i is automatically analyzed with the built-in software of the UF1000i and then classified into rods or cocci/mixed growth. Therefore, the application of the scatter diagram did not lead to additional analysis time or costs. Therefore, we recommended the routine use of scatter diagrams generated from UF-1000i as a diagnostic tool for uUTI because of significant difference in overall treatment success rate between rods and cocci/mixed groups

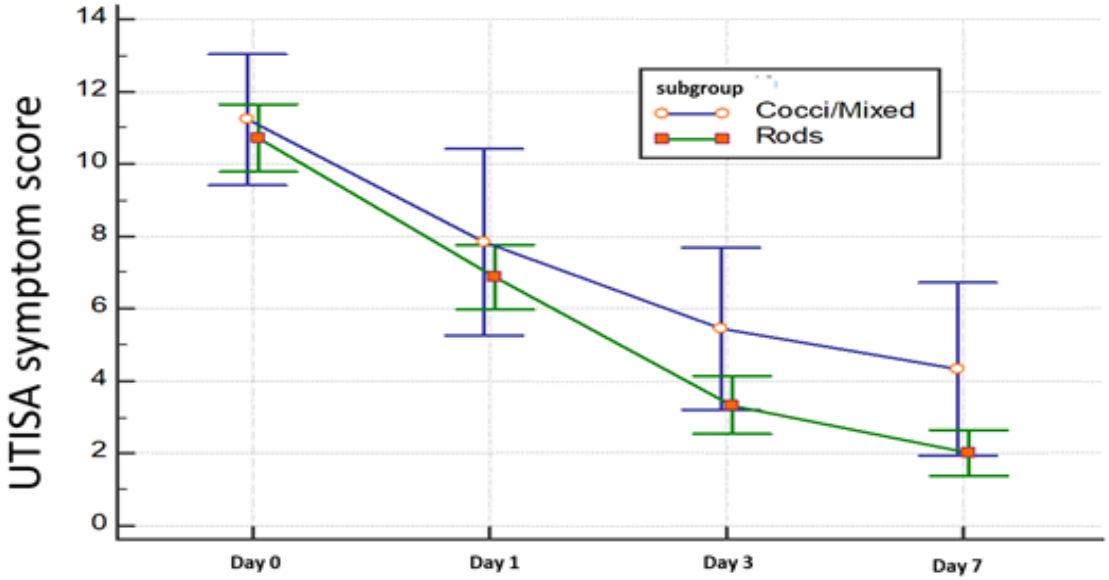

Figure 1. The change of symptom scores in subgroup patients.

Infectious Diseases Society of America (IDSA) and the European Society for Microbiology and Infectious Diseases suggest nitrofurantoin, Sulfamethoxazole / Trimethoprim and Fosfomycin as first-line antibiotics for management of uncomplicated symptomatic urinary tract infections based on the availability, allergy history and tolerance[12]. Since the resistance rate of the only available first-line antibiotics in Taiwan is Sulfamethoxazole/Trimethoprim which is associated with more than 20% resistance in our local area (supplement), we empirically used cephalexin for managing uUTI in community-dwelling women. The resistance rate of E. coli to cefazolin is 15% in our community patients (supplement) and the other community in Taiwan[13]. Overall, the symptom (UTISA < 4 om day 7) and laboratory improvement (bacteria count <100 bacteria/μL day 7) in patients treated with cephalexin were observed in 73 (73.7%) and 74 (74.7%) patients, respectively. The overall treatment success with both symptom and laboratory improvement was observed in 54 (54.5%) patients. The results supported the suggestions of IDSA that clinical cure (i.e. UTI symptom resolution) is expected within three to seven days after antibiotics treatment[12] (**Figure 1**). It is reasonable to repeat a urine tests if UTI symptoms persist beyond seven days. This will minimize unnecessary treatment of patients with persistent uUTI symptoms.

Urinary tract infection is ambiguously defined as lower urinary tract symptoms with positive urine culture without clear definition regarding the specific symptom and clear cut-off point for bacterial count(14). In this study, we adopted the criteria of UTISA >3 as positive symptoms and bacteria count >=100/μL as having bacteriuria[9]. UTISA > 3 is associated with a good sensitivity (87.0%) and specificity (93.1%) for differentiating women with uUTI from control(11). Empirical treatment with antibiotics in women with lower urinary tract symptoms may be not advised. A significant proportion (40/174, 23.0%) of women with symptoms suggestive of uUTI visiting urological clinics actually had low bacterial count which suggested that the symptoms may not be due to bacterial infection. Previous studies had demonstrated the efficacy of the UF-1000i in rapid diagnosis of bacteriuria. A meta-analysis reviewed the related studies to determine the capabilities of the UF-1000i in detecting bacteriuria. The pooled sensitivities were 87% and specificities were 60% for bacteriuria[15]. Various cutoff points of bacterial count had been reported in UTI screening and the variability could be attributed to different laboratories, patient populations, and the type of specimens. The bacterial scatter patterns generated from the bacterial laser diagram are classified when the bacteria count is > 100 BACT/microliter which is in line with the results of the study by De Rosa et al using bacterial count of 100/μL as cutoff point (Sensitivity: 96.5%, specificity: 86.7%,[9]) The usefulness of routine urine culture in female patients with uUTI has been questioned because voided urine culture may not perfectly reflect the true bacteriology in the bladder, especially for non-E coli related cystitis[16]. Since urine culture is expensive, labor-intensive, time-consuming, and prone to have false results, the UF-1000i provides cost-effective screening within 1 minute after urine sampling (100 samples/hour). In busy outpatient clinics, this means a real-time reporting that could reduce the blind initiation of antibiotics, and thus prevent unnecessary expenditure and drug treatment.

The analyzed specimens included 8 gram-positive bacteria (3 Streptococci spp., 3 Staphylococci spp., 1 Enterococci spp., and 1 Group B streptococci.), and 11 specimens with two bacteria species or more that were regarded as mixed growth. Since most cocci/mixed bacteria by the UF-1000i were skin flora or Gram bacteria resistant to cephalexin, avoiding use of antibiotics or choosing alternative antibiotics targeted at Gram positive bacteria may be helpful for clinical practice. However, the evidence supporting the use of alternative antibiotics require further studies.

There are several limitations to our study. Although the study is a prospective study evaluating the efficacy of the UF-1000i in predicting treatment outcomes of uUTI, a major limitation is that there was limited number of patient samples tested. The limited number of participants failed to show significant difference in UTISA scores improvement between rods and cocci/mixed gwoth cohorts. Despite the limited number of participants, there existed significant differences in treatment response between rods and cocci/mixed growth group. Second, the study was done at a single institution. Further studies are still warranted to evaluate the generalizability of the U-1000i in other institutions and populations. Further studies enrolling more patients in different areas with different antibiotics are warranted to prove the efficacy and exclude possible confounding factors. However, the strength of the study lies in that we used an objective parameter (bacterial count) and subjective symptom score (UTISA) at initial visit and follow up to validate the predictive ability of the U-1000i.

CONCLUSIONS

Women who had uUTI with urine samples exhibiting rods on automatic urine particle analyzer would have better treatment response to the use of first line antibiotics. Based on the current findings, the UF1000i can help clinicians pre-estimate the treatment response of uUTI in women.

CONFLICT ON INTEREST

The authors declare to have no conflict of interest.

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