Original Article

High False Positive Rate of White Blood Cells in Urine Samples of Pregnant Women may be Caused by Epithelial Cells Being Misclassified by the Sysmex UF-1000i Urine Flow Cytometer

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Background: The UF-1000i has been widely used in screening urinary sediments. However, the interference factor of the UF-1000i in the screening urinary sediments of pregnant women has not been reported. The aim of the study was to demonstrate that epithelial cells (ECs) cause a high false positive rate of white blood cells (WBCs) by the UF-1000i in pregnant women.

Methods: Urine samples were collected from 207 pregnant women. All samples were measured by the UF-1000i and a microscopic method.

Results: The areas under the curve (AUC) for WBC and EC counts were 0.837 (95% CI, 0.773–0.901) and 0.844 (95% CI, 0.785–0.903), respectively. The positive rates of the WBC and EC were 73.43% and 37.20%, respectively, by the UF-1000i, and they were 19.32% and 72.95% by the microscopic method. The positive predictive value, negative predictive value, false positive rates, and false negative rates by the UF-1000i were for WBC 25.66%, 98.18%, 74.34%, and 1.82%, respectively, and for EC they were 96.1%, 40.77%, 3.9%, and 59.23%, respectively. The coefficient of correlation *R* value was 0.503 ($P < 0.01$) between WBC by UF-1000i and EC by the microscopic method in WBC false positive samples.

Conclusions: EC could be an interference factor for the UF-1000i in screening urinary WBC of pregnant women, and the high false positive rate for WBC may be caused by ECs being misclassified as WBCs by the UF-1000i.

Key terms: epithelia; white blood cell; UF-1000i; misclassified; pregnant women

How to cite this article: Yang W, Yu X, Liu D, and Guan X. High False Positive Rate of White Blood Cells in Urine Samples of Pregnant Women may be Caused by Epithelial Cells Being Misclassified by the Sysmex UF-1000i Urine Flow Cytometer. Cytometry Part B 2019; 96B: 464–468.

INTRODUCTION

With the progress of science and technology, increasing areas of human labor have been taken over by machines, especially in the field of clinical laboratory. The last area where the machine cannot replace laboratorian seems to be microscopic morphology analysis, such as urinary sediment detection. The microscopic method is generally considered to be the gold standard in urine particle analysis (1,2). However, microscopic analysis is inefficient and has wide variability (3,4). In order to resolve this problem, flow cytometry has been utilized.

The UF-1000i is such a machine that uses fluorescence flow cytometry, has a red semiconductor laser (633 nm), and applies forward scatter(FSC) and side scatter (SSC) for size and granularity determination plus two fluorescence parameters to determinate

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Grant sponsor: National Natural Science Foundation of China, Grant number: 81672084

Received 22 May 2018; Revised 20 July 2018; Accepted 23 July 2018

Published online 10 October 2018 in Wiley Online Library (wileyonlinelibrary.com).

DOI: 10.1002/cyto.b.21727

urine red blood cells (RBCs), white blood cells (WBCs), epithelial cells (ECs), granulose cylinder, crystals, and bacteria (5,6).The analysis speed of UF-1000i is about 100 samples per hour, so large amounts of samples can be measured in a short time in the laboratory.

The analysis of urinary WBCs is very important for the diagnosis, differential diagnosis, treatment, and prognosis of diseases associated with the urinary system (7), particularly in combination with bacterial cultures to diagnose urinary tract infections (UTIs). Several studies had reported the reliability of UF-1000i for the detection of WBCs among outpatients with no pregnant women in community health clinics (8–10), but so far there has been no report about the analysis of WBC with UF-1000i in pregnant women. Perhaps their complexity restricts the study of urinary WBC of pregnant women. Interference factors include epithelia, large amounts of bacteria, and some impurities affect UF-1000i in screening for urinary WBCs, and in practice we noted that the epithelia seem to be a major interference factor in pregnant women.

In this study we compared the detection of WBC and EC by the UF-1000i with a microscopic method and aimed to demonstrate the reason why we had observed such a high false positive rate of WBC by the UF-1000i in pregnant women.

MATERIALS AND METHODS

Specimens' Collection

All pregnant women duration of 12–40 weeks gestation, free of nephropathy, diabetes, hypertension, and malignancies, for routine prenatal visits in the first affiliated hospital of Harbin Medical University, were enrolled between January 2017 and April 2017. Their ages varied from 20 to 44 years old (median = 31.75).

Random midstream urine was collected using a 12 mL sterile capped container. The pregnant women were excluded for the following reason: urine samples were not analyzed within 4 h.

UF-1000i Analysis

The equipment was calibrated and maintained daily according to the user manual. Internal quality controls were checked before the specimens were analyzed. The values of S-FSC, S-FSCW, S-FLH, S-FLL, S-SSC, B-FSC, and B-FLH for the Sysmex UF-1000i are 110.8, 28.2, 22, 141.8, 19, 92, and 118.4, respectively. All urine samples were analyzed for WBC and EC counts with the UF-1000i (Sysmex Corporation, Kobe, Japan) within an hour after collection, and the rest of each specimen was used for microscopic examination. Samples detected by the UF-1000i [cutoff of ≥16.9/μL for WBC and cutoff of ≥30.6/μL for EC] were considered as positive, otherwise as negative.

Microscopic Examination

We centrifuged the 10 mL urine specimens in a horizontal centrifuge at 400*g* for 5 min, discarded the supernatant urine, mixed the rest and then collected 0.2 mL of the urine sediment with a pipette and placed it on a glass slide, and then covered the urine sediment with an 18 × 18 mm coverslip. Under the light microscope, we counted the number of WBCs and ECs at high magnification for 10 consecutive visual fields and calculated the average number per visual field. Each sample was counted by two veteran morphologists under double-blind conditions. For the microscopic method the following diagnostic cut-off values of WBC and EC were used: negative: ≤5/HPF (high-power field); positive: >5/HPF.

Statistical Analyses

Statistical analysis was performed with SPSS 21 (SPSS, Inc., Chicago, USA). The microscopic method was considered as the gold standard in this study. The diagnostic performance of UF-1000i was assessed using receiver operating characteristic (ROC) curve analysis (11). Kappa tests were used to measure the actual agreement between the two morphologists and the two methods (UF1000i and microscopic method). The positive rate of the UF-1000i was compared with the positive rate of the microscopic method in screening WBC and EC using Pearson's chi-square tests. WBC results obtained from the UF-1000i and EC results obtained from the microscopic method in false positive WBC samples were compared using Pearson's correlation coefficient. For all analyses, $P < 0.05$ was defined as statistically significant.

RESULTS

Performance of UF-1000i in Screening Urinary WBC and EC Compared With the Microscopic Method

Microscopic method was chosen to be the gold standard. The ROC curves for WBC and EC counts from the UF-1000i are given in Figure 1. The areas under the curve (AUC) for WBC and EC counts were 0.837 (95%

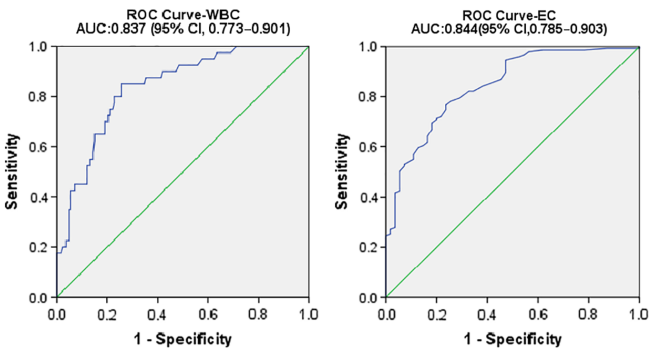

FIG. 1. Receiver operating characteristic (ROC) curve of WBC and EC counts on the UF-1000i. [Color figure can be viewed at wieyonlinelibrary.com]

Table 1
Comparison Between UF-1000i and the Microscopic Method in Urinary WBC and EC of Pregnant Women

	Positive samples by UF-1000i		Positive samples by microscopic method	
	n	%	*n*	%
WBC	152	73.43*	40	19.32*
EC	77	37.20**	151	72.95**

WBC, white blood cell; EC, epithelial cell.
*$P < 0.01$;
**$P < 0.01$

CI, 0.773–0.901) and 0.844 (95% CI, 0.785–0.903). An AUC between 0.7 and 0.9 is considered acceptable for a screening test in clinical practice, so the performance of the UF-1000i for WBC and EC was satisfactory. Kappa values between the two morphologists for WBC and EC are 0.94 and 0.956. It shows that the actual agreement between the two morphologists is very good. But the actual agreement between the two methods (UF-1000i and microscopic method) is poor. Their Kappa values of WBC and EC are 0.145 and 0.305.

Table 1 shows the comparison between UF-1000i and the microscopic method in screening urinary WBC and EC of pregnant women. During the study period, 207 urine samples were collected from pregnant women. The presence of WBC in urine measured by the UF-1000i was 73.43%, which is higher than their presence detected by the microscopic method. However, the positive rate of EC detected by the UF-1000i was lower than that detected by the microscopic method. The Pearson chi-square tests outcomes demonstrated a significant difference between the two methods in screening for WBC and EC ($P < 0.01$).

Tables 2–4 show further detailed performance of the UF-1000i and the microscopic method among pregnant women. For urinary WBC, there were only 39 true positive results in 152 positive results obtained by the UF-1000i. However, among 55 negative results there were 54 true negative results. For the urinary EC, 74 samples were true positive with only 3 false positive results, but in 130 negative samples, 77 samples were false negative. The positive predictive value for WBC was much less than the positive predictive value for EC. However, the negative predictive value for the WBC was significantly greater than that of the EC. The false negative rate for the WBC was only 1.82%, and the false positive rate was 3.9% for EC. The UF-1000i has a high sensitivity and low specificity in testing for WBC. However, for EC it is the opposite, with a low sensitivity and high specificity.

Correlations Between the UF-1000i WBC False Positives and the Respective Microscopic EC Counts

There were 97 EC positive samples tested by the microscopic method among the 113 WBC false positive samples measured by the UF-1000i. The positive rate of EC was 85.84% in WBC false positive samples. The correlation of urinary sediments counts between UF-1000i and microscopic method was calculated between the UF-1000i WBC false positives and the respective microscopic EC counts. The coefficient of correlation R value was 0.503 and $P < 0.01$. This showed that there was a significantly positive correlation between the UF-1000i WBC false positives and the respective microscopic EC counts (Fig. 2).

DISCUSSION

In our present study, we investigated the performance of the UF-1000i in screening urinary WBC and EC compared with the microscopic method in pregnant women. The ROC curves (Fig. 1) indicated that UF-1000i was satisfactory for this task. The AUC for WBC and EC counts were 0.837 (95% CI, 0.773–0.901) and 0.844 (95% CI, 0.785–0.903), which corresponds well with the previous studies (12,13). The microscopic method is considered to be the gold standard of urinary sediment analysis, but it is time-consuming (14). Because the number of urine samples is constantly increasing, the traditional method is increasingly unable to satisfy clinical laboratory needs. Simply considering the speed and the value of the AUC, the UF-1000i could be a good choice for urinary sediment analysis (15).

Nevertheless, after looking at the results of the positive predictive and negative predictive values in our study, we should pay more attention to the accuracy of the UF-1000i in screening for urinary WBC and EC.

Table 2
Results Tested by Two Methods in Urinary WBC of Pregnant Women

	Microscopic method		
UF-1000 i	+ (*n*)	– (*n*)	Total (*n*)
+	39	113	152
–	1	54	55
Total	40	167	207

Table 3
Results Tested by Two Methods in Urinary EC of Pregnant Women

	Microscopic method		
UF-1000i	+(*n*)	–(*n*)	Total (*n*)
+	74	3	77
–	77	53	130
Total	151	56	207

Table 4
The Performance of UF-1000i and Microscopic Method in Screening Urinary WBC and EC of Pregnant Women

	Positive results for both UF1000i and microscopic (*n*)	Negative results for both UF1000i and microscopic (*n*)	UF1000i positive/ microscopic negative (*n*)	UF1000i negative/ microscopic positive (*n*)	Positive predictive value (%)	False positive rate (%)	Negative predictive value (%)	False negative rate (%)
WBC	39	54	113	1	25.66	74.34	98.18	1.82
EC	74	53	3	77	96.1	3.9	40.77	59.23

At first, we were concerned with the parameter of WBCs. As demonstrated in Table 4, the negative predictive value was 98.18%. This shows that the UF-1000i has an almost perfect capability for diagnosing negative urinary WBCs in pregnant women. However, there were only 39 true positive results among the 152 positive results obtained by the UF-1000i, and the positive predictive value was only 25.66%. As a screening method, a positive predictive value of 25.66% is much too low to satisfy clinical needs. Although a high negative predictive value accompanied by a low positive predictive value could be preferable to increase the cell count accuracy (16), the false positive rate of 74.34% will require a lot of effort to recheck these results and therefore the workload will not be reduced by using the UF-1000i. Second, the parameter of EC also needs to be noticed. Table 4 shows that the UF-1000i has a high positive predictive value and low negative predictive value for EC, which is the opposite for its results for WBC. The UF-1000i with its high positive predictive value could reduce the rates of microscopic rechecking, but a false negative rate of 59.23% is not satisfactory. This means that there will be 59.23% of positive samples misjudged as negative samples. Therefore, the high false positive rate for WBC and high false negative rate for EC might be a bottleneck in the application of the UF-1000i in screening WBC and EC for pregnant women.

To investigate the reason why there existed such a high false positive rate for WBC and a high false negative rate for EC, we reviewed by the microscopic method 113 false positive results of WBC detected by the UF-1000i. We found that there were 97 EC positive results among the113 WBC false positive results, so we conducted a correlation analysis between the WBC results by the UF-1000i and the EC results by the microscopic method among the 113 WBC false positive results. The results of the correlation analysis ($R = 0.503$, $P < 0.001$) showed that there was a significantly positive correlation between WBC and EC in WBC false positive samples. Thus, we speculated that the EC might interfere with the UF-1000i in screening for urinary WBC, and some EC might be misjudged as WBC by the UF-1000i. This is probably why the UF-1000i has a high false positive rate for WBC and a high false negative rate for EC in screening urinary sediments of pregnant women.

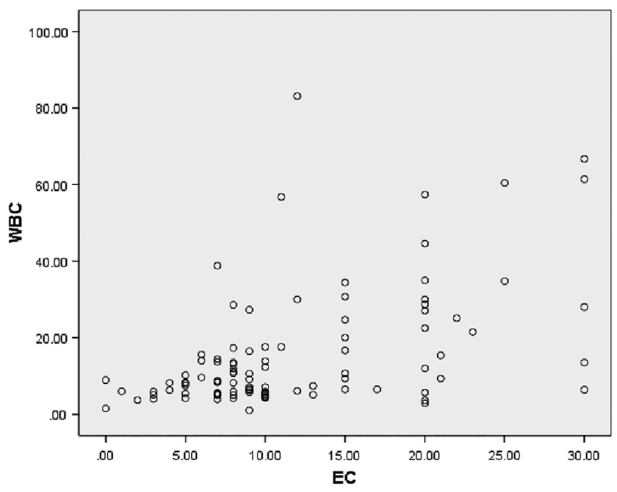

FIG. 2. Correlations between the UF-1000i WBC false positives and the respective microscopic EC counts ($R = 0.503$, $P < 0.01$)

The UF-1000i utilizes an optical signal bandwidth for the classification of urine particles. On the UF-1000i, the FSC, SSC, and fluorescence intensity (FI) signals provided the best classification of WBC and RBC. The diameter of a WBC is larger than a RBC generally and granules of WBC are more than that of RBC, plus WBCs have a nucleus and RBCs do not, and a nucleus can emit FI when it passes through the inspection port of the UF-1000i, so the signals of FSC, SSC, and FI for WBC are much stronger that for RBC. However, these signals are inadequate to differentiate WBC and EC because the configuration of WBC and EC are similar. Both WBC and EC have a structure of cytomembrane, cytoplasm, and nucleus. The diameter of some ECs is approximately the same as the diameter of WBCs; granules of EC and WBC are not significantly different, and both EC and WBC can emit strong FI signals. Therefore, just based on the signals of FSC, SSC, and FI, they are hard to distinguish by the UF-1000i. In order to improve the detection rate of WBCs, the UF-1000i would need to extend the signal band width for detecting WBC. Thus, some EC might be misjudged as WBC and the phenomena would become more serious as the number of EC increases. Under normal conditions, there are few EC in a urine sample, and the interference of EC can be ignored. However, in pregnant women urine, the number of EC increases significantly (17), and many ECs are misclassified as WBCs by the UF-1000i. That is why there are such a high false positive rate of WBC and a high false negative rate of EC by the UF-1000i for pregnant women.

In this study, we showed for the first time that in pregnant women urine, EC was an interference factor for the

UF-1000i in screening urinary WBC, and the UF-1000i misjudging EC as WBC caused a high false positive rate of WBC and a high false negative rate of EC.

The limitations of our study are that there were only 207 samples in this study, and all samples were collected from outpatients. Therefore, our results may not represent all pregnant women, and further research is needed.

In conclusion, EC could be an interference factor for the UF-1000i in screening urinary WBC of pregnant women, and the high false positive rate of WBC for the UF-1000i may be caused by EC being misclassified as WBC.

ACKNOWLEDGMENTS

The present study was supported by the National Natural Science Foundation of China (81672084). We are extremely grateful to clinical laboratory staffs who helped in this study.

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