Renal tubular epithelial cells add value in the diagnosis of upper urinary tract pathology

https://doi.org/10.1515/cclm-2019-1068
Received October 15, 2019; accepted November 23, 2019

Abstract

Background: Diagnosis of upper urinary tract infections (UTI) is challenging. We evaluated the analytical and diagnostic performance characteristics of renal tubular epithelial cells (RTECs) and transitional epithelial cells (TECs) on the Sysmex UF-5000 urine sediment analyzer.

Methods: Urinary samples from 506 patients presenting with symptoms of a UTI were collected. Only samples for which a urinary culture was available were included. Analytical (imprecision, accuracy, stability and correlation with manual microscopy) and diagnostic performance (sensitivity and specificity) were evaluated.

Results: The Sysmex UF-5000 demonstrated a good analytical performance. Depending on the storage time, storage conditions (2–8 °C or 20–25 °C) and urinary pH, RTECs and TECs were stable in urine for at least 4 h. Using Passing-Bablok and Bland-Altman analysis, an acceptable agreement was observed between the manual and automated methods. Compared to TECs, RTECs demonstrated an acceptable diagnostic performance for the diagnosis of upper UTI.

Conclusions: While TECs do not seem to serve as a helpful marker, increased urinary levels of RTECs add value in the diagnosis of upper UTI and may be helpful in the discrimination between upper and lower UTIs.

Keywords: automated urinary sediment analyzer; renal tubular epithelial cells; urinary tract infections.

Introduction

Differentiation between lower and upper urinary tract infections (UTI) is of paramount clinical importance, for which clinicians often have to rely on clinical symptoms. However, in adults and children, clinical criteria do not allow a 100% discrimination between both pathologies [1, 2]. Also, current laboratory methods do not always allow a clear-cut distinction between upper UTI and lower UTI (LUTI). Microbiological culture is not helpful in this respect as it does not provide information regarding the localization of the infection. Therefore, differentiating LUTI from upper UTI remains a diagnostic challenge in modern urinalysis. Urine sediment analysis can be helpful: the presence of leukocyte casts in the urine sediment is suggestive of an upper UTI, but its diagnostic sensitivity remains low [3]. Specific urinary proteins (e.g. α1-microglobulin) are established markers of tubular function, which have been shown to be helpful to some extent [4]. However, these markers may not be available in every routine clinical laboratory.

The Sysmex UF-5000 (Sysmex Corporation, Kobe, Japan) represents the newest generation of urinary particle analyzers, designed for automated urine sediment and body fluid analysis. This third-generation fluorescence flow cytometry analyzer for urinalysis offers the enhanced possibility to accurately count and differentiate a broad variety of urinary cells. The instrument is able to differentiate epithelial cells into squamous (SEC), transitional (TECs) and renal tubular epithelial cells (RTECs). RTECs along with TECs line the urinary tract, originate from the proximal or distal urine segments and therefore have a diagnostic potential [5], whereas high SEC counts might point toward contaminated urine and add only little diagnostic value. RTECs are specific for the presence of tubular damage and their detection in a urinary sample could allow early recognition of renal damage when other kidney function parameters are still normal [5, 6]. On the contrary, TECs are markers of ureteral damage related to the presence of an infection, kidney stones or invasive procedures [5]. As these types of cells may differ in morphological presentation, they are a diagnostic challenge in modern clinical urinalysis laboratories [6].
The aim of the present study was to explore the analytical performance and diagnostic value of RTECs and TECs in a cohort of well-documented patients presenting with symptoms of a UTI. In particular, we aimed to determine the value of RTECs and TECs for the diagnosis of upper UTI. Furthermore, we wanted to compare the diagnostic performance of RTECs and TECs with that of some established upper UTI markers (α1-microglobulin, pathological casts, γ-glutamyl transpeptidase [GGT] activity).

Materials and methods

Patient population

The total population consisted of 506 patients (median age: 58 years [range: 18–97 years]; males: n = 237; females: n = 269) with a suspicion of a UTI and for whom a midstream urine sample for test strip and sediment analysis was sent to the clinical laboratory of the Ghent University Hospital. Based on the final diagnosis made by the clinician, patients were retrospectively categorized into upper UTI (pyelonephritis [n = 73], urosepsis [n = 32] and renal cystic infection [n = 2]), LUTI (cystitis [n = 35] and prostatitis [n = 12]) and 352 patients without UTI were categorized as disease controls. The main reasons for performing urine sediment analysis were suspected clinical symptoms of a UTI (pollakisuria, dysuria, abdominal or flank pain, fever chills, etc.). Only patients with a proven microbiological UTI were included. A summary of the patient characteristics along with the RTEC and TEC results of the Sysmex UF-5000 is presented in Table 1. The exclusion criteria were an unclear diagnosis, the inability to collect enough urine and an age <18 years.

Urine samples were collected by the aspiration technique using Sarstedt Monovette urinary collection tubes (Sarstedt, Numbrecht, Germany) and were processed within 2–4 h after arrival in the laboratory. Test strip analysis on a Sysmex UC-3500 analyzer (Sysmex, Kobe, Japan) [7] was carried out before flow cytometry analysis on the Sysmex UF-5000 (Sysmex, Kobe, Japan). After microscopic analysis, urinary chemistry tests (see further) were determined. The study was performed with the full respect for individuals’ rights to confidentiality and in accordance with procedures supervised by local authorities responsible for ethical research (Belgian registration number of ethical approval: B670201837110).

Urine particle analysis using UF-5000 and microscopic analysis

Measurement of RTECs and TECs was performed using the Sysmex UF-5000 (Sysmex, Kobe, Japan). The UF-5000 is able to recognize, count and classify cells by analyzing forward scatter light, side scatter light, side fluorescent light and the depolarized side scattered light of stained particles. Depolarized side scattered light was introduced to improve the sensitivity of crystals and to better discriminate red blood cells (RBCs) from crystals. The principle is based on a 488-nm blue laser flow cytometry. The UF-5000 measures urinary conductivity and categorizes the particles based on their size, intracellular structure and staining characteristics. The signals are displayed in scattergrams and histograms and the results are given as counts per μL as well as counts per high power field (HPF) [8]. The UF-5000 automatically detects and counts RBCs, non-lysed RBCs, white blood cells (WBCs), WBC clumps, bacteria, yeast-like cells, crystals, RTECs, TECs, sperm cells and (hyaline and pathological) casts. Urinary particles that cannot be classified into one of the former categories are counted as “other cells”.

Following the analysis on the automated urine sediment analyzer, microscopic identification and counting were performed by phase-contrast microscopy on uncentrifuged urine samples using disposable Uriglass counting chambers (A. Menarini Diagnostics, Florence, Italy) by two expert laboratory technicians blinded to the results of the Sysmex UF-5000. Each 1 μL chamber is divided into 10 large squares. Each square corresponds to a volume of 0.1 μL and is subdivided into 16 small squares. RTECs and TECs were counted in 5 x 16 squares, and the results are expressed as the number of particles per μL of urine.

Biochemical investigations

Following the urinary sediment analysis, biochemical investigations were also performed. Total protein was determined on all samples by a pyrogallol red-molybdate method (Instruchemie BV, Delfzijl, The Netherlands) [9]. Urinary α1-microglobulin was assayed immunoturbidimetrically using Roche reagents (Mannheim, Germany). Urinary GGT activity was measured according to the method of Szasz [10] using commercial reagents from Abbott. Urinary creatinine and urinary urea were also determined using Abbott commercial reagents. All analyses were performed on the Abbott Allinity C analyzer (Abbott Diagnostics, Wiesbaden, Germany).

Table 1: Overview of the patient characteristics and RTEC and TEC counts (/μL) of the different patient groups.

<table>
<thead>
<tr>
<th>Final diagnosis</th>
<th>n</th>
<th>Men/women, n/n</th>
<th>Age, years (range)a</th>
<th>RTECs, /μLb</th>
<th>TECs, /μLb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper urinary tract infection</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urosepsis</td>
<td>32</td>
<td>19/13</td>
<td>71 (27–93)</td>
<td>5.5 (3.3–10.6)</td>
<td>0.6 (0.1–1.3)</td>
</tr>
<tr>
<td>Pyelonephritis</td>
<td>73</td>
<td>19/54</td>
<td>59 (18–97)</td>
<td>9.8 (6.3–13.6)</td>
<td>0.6 (0.3–1.3)</td>
</tr>
<tr>
<td>Lower urinary tract infection</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cystitis</td>
<td>35</td>
<td>6/29</td>
<td>58 (18–95)</td>
<td>1.4 (0.3–4.3)</td>
<td>0.3 (0.1–0.9)</td>
</tr>
<tr>
<td>Prostatitis</td>
<td>12</td>
<td>12/0</td>
<td>69 (60–85)</td>
<td>1.5 (0.7–2.1)</td>
<td>0.7 (0.0–2.1)</td>
</tr>
<tr>
<td>Non-urological/non-nephrological patients</td>
<td>354</td>
<td>181/173</td>
<td>55 (18–94)</td>
<td>1.3 (0.4–2.6)</td>
<td>0.1 (0.0–0.3)</td>
</tr>
</tbody>
</table>

aAge is presented as years (range). bThe value within brackets indicates the interquartile range (IQR). RTECs: renal tubular epithelial cells; TECs: transitional epithelial cells.
Microbiological culture

Biplates with Tryptic Soy Agar (TSA) + 5% sheep blood and MacConkey agar (Becton Dickinson, Cockeysville, MD, USA) were used for chairside inoculation of 1 μL of freshly voided urine, and incubated upon arrival at 35 °C ambient atmosphere overnight and for another 24 h. Interpretation is done after 24 and 48 h of incubation. All suspect colony types were identified using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) (Microflex LT, Bruker, Billerica, MA, USA). Criteria for positivity of a urine culture were in accordance with the European Federation for Urinalysis guidelines [11].

Statistical analysis

Imprecision of RTECs and TECs on the UF-5000 was assessed using three patient samples (low, medium and high level). Intra-run imprecision was determined by measuring each sample 10 times in the same run. As the stability of RTECs is limited, only within-run imprecision was assessed. Bias was determined by calculating the % difference between the mean RTEC value of the intra-run imprecision experiment and the target value. This target value was determined by manually counting the number of RTECs and TECs. The total error (TE) was calculated using the formula: TE = bias + 1.65 * (intra-run imprecision [%]).

The stability of RTECs and TECs at two different storage conditions (room temperature [20–25 °C, 24 h] and refrigerator [2–8 °C, 24 h]) on 10 different samples was determined (RTEC: mean concentration: 21.7/μL, range: 9.3–38.9/μL; TEC: mean concentration: 17.4/μL, range: 5.8–55.4/μL). Samples were determined 2, 4, 6, 8, 10, 12, 16 and 24 h after the initial measurement. Allowable TE internal laboratory criteria for SECs (i.e. 30%) were used as criterion for significant deviation with respect to the initial value. RTEC and TEC concentrations were determined using the UF-5000.

For analytical method comparison, Bland-Altman plots, Passing-Bablok regression analysis and Spearman’s rank correlation coefficients were determined for both assays.

Differences in RTEC count between (sub)groups were assessed for significance using the Mann-Whitney U test. p-Values <0.05 were considered statistically significant. Receiver operating characteristic (ROC) curve analysis and areas under the ROC curve (AUC) were calculated to evaluate the diagnostic accuracy of the different parameters measured. Sensitivities and specificities were determined with the clinical diagnosis. Optimal cut-offs were defined as cut-off values with the highest sum of sensitivity and specificity, based on ROC curve analysis.

Statistical analysis was performed using the MedCalc software (version 15.6.1., Mariakerke, Belgium).

Results

Analytical performance

Imprecision, bias, total error and stability

Within-run imprecision ranged from 6.0 to 10.6% and 5.0 to 12.9% for RTECs and TECs, respectively. The bias relative to the manual count ranged from −2.6 to 6.5% and −5.9 to 15.2% for RTECs and TECs, respectively. The calculated TE was acceptable and ranged from 12.5 to 24.0% for RTECs and 9.8 to 36.5% for TECs. A summary is presented in Table 2.

We determined the stability of RTECs and TECs at both room temperature (20–25 °C) and in the refrigerator (2–8 °C) and found that the stability of both parameters depended on the pH of the urinary sample. RTEC and TEC counts in samples with a pH ≥7.5 were stable for maximum 4 h at both room and refrigerator temperature. RTEC and TEC counts in samples with a pH <7.5 were stable for 8 h at room temperature. In the refrigerator, TEC counts were stable for 12 h and RTECs for 16 h. The results are presented in Figure 1.

Table 2: Analytical performance (imprecision, bias and total error) of RTECs and TECs on the Sysmex UF-5000.

<table>
<thead>
<tr>
<th></th>
<th>Imprecision (within-run)</th>
<th>Bias</th>
<th>Total error</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean, /μL</td>
<td>SD, /μL</td>
<td>CV, %</td>
</tr>
<tr>
<td>RTECs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>5.6</td>
<td>0.6</td>
<td>10.6</td>
</tr>
<tr>
<td>Medium</td>
<td>16.4</td>
<td>1.4</td>
<td>8.7</td>
</tr>
<tr>
<td>High</td>
<td>39.0</td>
<td>2.3</td>
<td>6.0</td>
</tr>
<tr>
<td>TECs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>2.5</td>
<td>0.3</td>
<td>12.9</td>
</tr>
<tr>
<td>Medium</td>
<td>20.6</td>
<td>1.4</td>
<td>7.1</td>
</tr>
<tr>
<td>High</td>
<td>49.8</td>
<td>2.5</td>
<td>5.0</td>
</tr>
</tbody>
</table>

SD, standard deviation; CV, coefficient of variation; RTECs, renal tubular epithelial cells; TECs, transitional epithelial cells. *Source criteria: internal laboratory criteria.
Method comparison

Method comparison was performed on 504 samples. Passing-Bablok regression analysis between manual microscopic and automated RTEC counts revealed a proportional difference of approximately 4% (slope: 0.96 [95% CI: 0.91–1.00]; intercept: −0.42 [95% CI: −0.50 to −0.36]; R²: 0.972 [95% CI: 0.966–0.976]) (Figure 2A1). Using Bland-Altman analysis, a mean difference of −10.1 RTEC cells/μL (95% CI: −16.0 to −4.2/μL) was obtained.
with limits of agreement (LOA) of −104.7 cells/μL (95% CI: −114.8 to −94.6) and 84.5 cells/μL (95% CI: 74.4–94.6) (Figure 2B1).

For TEC, no significant proportional or systemic differences were obtained between manual and automated counts (slope: 1.00 [95% CI: 0.90–1.30]; intercept: 0.30 [95% CI: 0.30–0.30]; R²: 0.980 [95% CI: 0.977–0.984]) (Figure 2A2). Bland-Altman analysis revealed a mean difference of −2.3 TEC cells/μL (95% CI: −8.1 to 3.6) with LOA of −51.1 (95% CI: −61.1 to −41.0) and 46.5 (95% CI: 36.5–56.6) (Figure 2B2).

Correlation study

Multiple linear mixed model analysis revealed significant correlations between RTECs and log-transformed GGT (β = 3.1865, standard error [SE] = 0.648, p < 0.0001), completed by log-transformed bacteria (β = 1.1091, SE = 0.207, p < 0.0001) and α₁-microglobulin (β = 0.0269, SE = 0.007, p < 0.0001). The R² value of the final model was poor (0.167). No significant correlations were obtained between TECs and the other measured urinary parameters.

Diagnostic performance

Among the study population, the prevalence of upper UTI was 21.1% (107/506). Quantitative data distribution showed that RTEC counts were significantly higher (p < 0.05) in patients with a final diagnosis of urosepsis and pyelonephritis compared to counts in patients diagnosed with cystitis and prostatitis (see Figure 3). On the contrary, no significant difference in TECs between the different subgroups was observed.

In our population, the diagnostic accuracy (expressed as AUC) for the parameters α₁-microglobulin, bacteria, GGT, WBCs, RTECs, TECs and pathological casts on the Sysmex UF-5000 was found to be 0.735 (95% CI: 0.694–0.773), 0.787 (95% CI: 0.748–0.821), 0.586 (95% CI: 0.541–0.629), 0.816 (95% CI: 0.779–0.849), 0.923 (95% CI: 0.897–0.945), 0.790 (95% CI: 0.752–0.825) and 0.751 (95% CI: 0.711–0.788), respectively (see Figure 4). The AUCs were statistically significantly different (p < 0.05) between RTECs and α₁-microglobulin, bacteria, GGT, pathological casts, TECs and WBCs. Statistically significant differences in AUCs were also observed between α₁-microglobulin and WBCs and pathological casts and WBCs (p-value <0.01). A summary of the diagnostic performance of these parameters measured on the Sysmex UF-5000 in our study population is presented in Table 3. The same AUCs were demonstrated for RTECs and TECs counted by manual microscopy (data not shown).

The median conductivity and urinary creatinine concentrations of the samples in the study were 13.2 mS/cm (interquartile range [IQR]: 9.2–18.6 mS/cm) and 91.0 mg/dL (IQR: 48.7–140.9 mg/dL), respectively. The obtained diagnostic performance (expressed as AUC) for RTECs corrected for conductivity and RTECs corrected for urinary creatinine (0.900 [95% CI: 0.867–0.927] and 0.905

![Figure 3: Box-whisker plots representing renal tubular epithelial cell (RTEC) counts among the different clinical conditions. Values falling outside the box-whisker plot are outliers.](image)

![Figure 4: Presentation of the receiver operating characteristic (ROC) curve comparison for the parameters α₁-microglobulin, urinary bacterial count, white blood cells (WBCs), renal tubular epithelial cells (RTECs), tubular epithelial cells (TECs) and pathological casts.](image)
Oyaert et al.: Added value of RTECs in UTI

[95% CI: 0.872–0.931], respectively) was not significantly different compared to the AUC of RTECs (AUC: 0.911 [95% CI: 0.880–0.936]), demonstrating that there is no added value when the results are corrected for dilution parameters.

**Discussion**

While SECs and TECs offer little useful information, we have demonstrated for the first time that RTECs can be regarded as an interesting supplemental parameter in the discrimination between upper UTI and LUTI. In line with previous studies [8, 12], our study showed that the Sysmex UF-5000 urine sediment analyzer is able to count the RTECs and TECs with an acceptable analytical performance. These parameters were not available on earlier generations of urinary flow cytometers [3, 13, 14], categorizing RTECs as “small round cells”. Compared to manual microscopy, a sensitivity and a specificity of 95% and 75% for RTECs and 71% and 94% for TECs at a cut-off of 3 and 5 cells/μL were reported, respectively [8]. Due to the improved categorization of urinary particles of the UF-5000, the discriminatory power of this new-generation urinary flow cytometer with respect to localization of UTI has much improved.

Multiple regression analysis revealed that RTEC values strongly correlated with bacterial and WBC counts, which are indicators of UTI. Similarly, a strong correlation was found between RTEC count and urinary α1-microglobulin concentration and GGT activity in urine. The latter two parameters have been previously described as indicators of upper UTI [3, 15].

As expected [5], increased RTEC counts were observed in urine specimens from patients with upper urinary tract pathology. Inflammation is known to target TECs. Tubular cells have been implicated in the response to inflammatory mediators in ischemic and septic renal damage [19].
We demonstrated that RTECs may add value in the discrimination between upper UTI and LUTI. At a cut-off level of 3.1 cells/μL, which corresponds with the manufacturer’s set upper reference limit (0–3 cells/μL), we found a sensitivity and a specificity of 93.5% and 82.2%, respectively. The diagnostic performance of RTECs in the differentiation between upper UTI and LUTI exceeded by far the one of TECs.

In our previous study, α1-microglobulin proved to be a useful discriminator between upper UTI and LUTI [3, 20]. However in this study, comparative ROC analysis demonstrated that the diagnostic utility of RTECs for detecting upper UTI outperforms that of α1-microglobulin, GGT activity, leukocyturia and pathological casts. In accordance with the findings of Penders et al. [3], we found that pathological casts showed a low specificity for localizing UTI. These findings could be attributed to the low number of casts usually found in urinary specimens and the difficult quantification of these urinary structures in urinary particle analysis [13, 21, 22].

Time between sampling and performance of the examination procedure is critical for the reliability of urinary test results [23]. Stability data are known for most usual urine sediment parameters [24], but lack for RTECs and TECs. We found that the stability of RTECs and TECs at room temperature is limited to 4 h. As most clinical laboratories are able to process urinary samples for urinalysis immediately after arrival in the laboratory, this limited stability can be overcome. However, special attention should be paid to the pH dependent stability of RTECs and TECs, as a higher pH (pH > 7.5) decreases the RTEC and TEC stability with approximately 8 h. The same dependency has been observed for urinary casts and WBCs [25], which are also lost in alkaline urines. High urinary pH values may be observed in infections with urease-producing bacteria [25]. Cooling of the specimen at 2–8 °C improves the stability of both parameters.

Although the inbuilt UF-5000 osmolality parameters may allow to correct for urinary dilution, the diagnostic power of RTECs cannot further be improved by correcting results using urinary conductivity and urinary creatinine. This could be explained by the fact that the stability of both parameters is, as urinary WBCs, affected by extremely diluted or concentrated specimens [25].

In conclusion, our study adds interesting information on the performance of some new parameters that recently have become available on a routine new generation urine sediment analyzer [26]. Along with the results of urinary microbiological culture and clinical evaluation, RTECs may be considered as an interesting supplemental analyte for differentiating upper UTI from LUTI.

**Author contributions:** All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

**Research funding:** None declared.

**Employment or leadership:** None declared.

**Honorarium:** None declared.

**Competing interests:** The funding organization(s) played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

**References**