Assessment of haematuria: automated urine flowmetry vs microscopy

Terje Apleand¹, Oddvar Mestad² and Øyvind Hetland³

¹Department of Medicine, ²Department of Surgery and ³Department of Clinical Chemistry, Rogaland Central Hospital, Stavanger, Norway

Abstract

**Background.** Microscopy of the urine sediment may be a useful method in the distinction between a glomerular and a non-glomerular source of urinary bleeding. However, microscopic techniques are time consuming and hampered by inter-observer variations. In the present study we have therefore compared bright-field microscopy with automated urine flowmetry (Sysmex® UF-100), examining their ability to differentiate between glomerular and non-glomerular haematuria.

**Methods.** Fresh urine samples were obtained from 112 patients with a well-defined, single cause of a positive dipstick test. Their urine specimens were examined within 4 h in a blinded manner. Of them, 79 specimens had a positive dipstick for blood and thus could be evaluated for haematuria.

**Results.** The Sysmex® UF-100 had a sensitivity and specificity of 0.83 and 0.94 respectively in detecting non-glomerular bleeding. The positive and negative predictive values were 0.95 and 0.78 respectively. The corresponding values of microscopy were 0.79 and 0.90 respectively, and 0.93 and 0.74 respectively.

**Conclusions.** Automated flowmetry can be used in the distinction between glomerular and non-glomerular haematuria.

**Keywords:** glomerular; haematuria; non-glomerular; urine flowmetry; urine microscopy; urine sediment

Introduction

The morphological differences between the dysmorphic erythrocytes from a glomerular and the isomorphic erythrocytes from a non-glomerular source of bleeding, have been described [1–4]. The causes of red-cell dysmorphism are not fully known. It has been suggested that the altered shape and variable size of erythrocytes from pathological nephrons are the result of a dual injury: (i) haemolytic injury when entering the nephron, and (ii) exposure within the tubular lumen to osmotic forces [5–7]. Persistent symptomless microscopic haematuria is a common condition [8]. Among patients over the age of 50 years, more than 10% of their urine samples may have a positive dipstick for blood [9]. The large number of asymptomatic patients render an efficient plan necessary before clinical evaluation. However, this may be difficult as both urologists and nephrologists are usually involved. An examination of the urine may be helpful when deciding where to begin—at either the urological or the nephrological clinic—thereby reducing the number of unnecessary investigations. Microscopic assessment of urinary erythrocyte morphology has been shown to be useful in the management of patients with symptomless microscopic haematuria [10–12]. However, microscopy of the urine is time consuming and is hampered by inter-observer variations [13]. In the past, flowmetric analysers originally designed for evaluation of venous blood were employed to run urine samples [14,15]. This proved to be difficult and cumbersome [16–18]. However, after the introduction of the automated urine flowmetry (Sysmex® UF-100) it became easier to perform accurate counts of the formed elements of the urine [19–23]. Furthermore, automated flow cytometry provides a rapid and objective assessment of urinary erythrocyte size. On the other hand, flowmetry cannot identify the altered shapes of dysmorphic erythrocytes or identify erythrocyte casts [21,23]. Thus, urine microscopy and flowmetry are encumbered with advantages and disadvantages. Nevertheless, we found it essential to examine which of the two methods was most appropriate in the distinction between glomerular and non-glomerular haematuria from unselected patients in a clinical setting. To our knowledge, this has not been done previously.

Subjects and methods

Patients from the nephrological ward, urological ward, and outpatient clinics were invited to participate in the study.
Patients with a kidney transplant or a known urinary infection were excluded. After giving their informed consent, 129 patients delivered a freshly voided urine sample during working hours at the laboratory. Seventeen patients were excluded as they had no definite diagnosis or had two or more potential sources of bleeding. The remaining 112 patients, 26 female and 86 male patients, were included. Fifty-seven had a nephrological disease and 55 had a urological disorder. The mean age of those with a nephrological disease was 51.2 ± 16.5 (23–81) and for those with a urological disorder 69.8 ± 16.8 (23–81) (mean ± standard deviation, range, years). Patients with a proven neoplasms (n = 30) had an average age of 75.8 ± 11.8 (40–91).

The urine samples were immediately cooled with laboratory numbers. All information about subject identity was removed. The code was broken after all urine samples were examined. Within 4 h, three tests were run on the samples: (1) reagent strip testing with Multistix 8 SG® (Bayer, USA) and a dipstick reader, Clinitek 200® (Ames Division, Miles laboratories Inc, Elkhart, Indiana, USA). (2) After the centrifugation of 10 ml of urine to 1000 r.p.m. for 3 min, the supernatant was discarded and a drop of Sterneheimer–Malbin stain added to the urine sediment. An experienced nephrologist examined the preparations under a conventional bright-field microscope (Olympus BH-2) at 100 × and 400 × magnification. (3) Examination of 10 ml of urine with the automated urine cell analyzer UF-100 (Sysmex UF-100, Sysmex GMBH Europe, Hamburg, Germany). The printed results of the urine flowmetry were evaluated blindly, at the end of the study. The patient records and their diagnosis were evaluated separately, without any knowledge of the results of urine analysis.

The Sysmex UF-100 employs flowmetry and impedance detection in order to identify and count urine formed elements. Native urine is automatically aspirated into the system, diluted and stained with two fluorescent dyes: phenoanthridine, which stains nucleic acids, and carboxycyanine, which stains the cytoplasmic reticulum. The elements of the urine pass through an argon laser beam and between a pair of electrodes, while scattered light, fluorescence, and impedance for each particle are recorded and digitally processed. The five basic parameters presented quantitatively are the number of erythrocytes, leukocytes, epithelial cells, casts, and bacteria. The particle counts have a low within-run imprecision, ranging from 17.7 to 2.4% and a good and linear correlation with the results obtained by urine sediment microscopy [23]. Furthermore, the presence of abnormal high levels of yeasts, crystals, pathological casts, small round cells (i.e. mostly renal tubular cells), and spermatozoa are identified. Lastly, the volume histograms of erythrocytes and leukocytes are provided (Figure 1). The UF-100 system can process up to 100 urine specimens in an hour.

The Kitasato criteria were the major criteria applied to differentiate between a glomerular and a non-glomerular pattern of the erythrocyte volume histogram [24,25]. Then, the glomerular discrimination point for erythrocyte volume is defined as ≤126 channels (ch) and the non-glomerular discrimination point is ≥84 ch (i.e. erythrocyte diameter of 6 and 4 μm respectively). If more than 80% of erythrocyte volumes are ≤126 ch and less than 80% ≥84 ch, the haematuria is regarded as glomerular (Figure 1A). If more than 80% of erythrocyte volumes are ≥84 ch, the haematuria is regarded as non-glomerular (Figure 1B). If less than 80% of erythrocyte volumes are ≤126 ch and less than 80% ≥84 ch, the haematuria is regarded as mixed.

When the erythrocyte findings were ambiguous (i.e. mixed haematuria or doubtful quality of erythrocyte volume histogram) minor criteria were employed to identify the source of haematuria. The presence of high levels of leukocytes, bacteria, or yeast indicated a non-glomerular disorder, while the presence of increased numbers of small round cells (i.e. mostly renal tubular cells) or pathological casts made a glomerular bleeding more likely. In the same way, all information from the microscopic examination of urine sediments was combined to make a decision about the type of urinary bleeding.

Statistical calculations were performed using StatView 5.0 for Power PC (SAS Institute Inc. 1998, Cary, North Carolina, USA). The chi-squared test was used for categorical differences. The cut-off level for statistical significance was set at $P \leq 0.05$.

**Results**

One hundred and twelve patients were included in the study. Seventeen patients had a negative urinary dipstick reading for both blood and albumin. Sixteen patients had a positive reading for albumin and a negative reading for blood. The urine specimens from the remaining 79 patients were dipstick positive for blood and thus suited for evaluation of erythrocyte morphology. By the urinary dipstick testing for blood, 14 patients had 1+ haematuria, 32 had 2+ haematuria, and 33 had 3+ haematuria (Table 1).

Urine flowmetry correctly identified 29 of 31 patients with nephrological disease and 40 of 48 patients with a urological disease ($P < 0.0001$, Table 2). Thus, urine flowmetry in patients with a positive dipstick for blood had a sensitivity of 0.83 and a specificity of 0.94 in detecting a non-glomerular bleeding. The positive and negative predictive values were 0.95 and 0.78 respectively (Table 1).

Eight patients had a mixed type of urinary erythrocyte volume histogram, and by means of the minor criteria, a urological source of the urinary bleeding was correctly identified in all eight patients.

Urine flowmetry gave an incorrect diagnosis in two patients with a nephrological disease (Table 2). Both
had urine samples with low erythrocyte counts (about 1 per high-power field (×400) (HPF)).

Eight patients with urological disorders were incorrectly classified by the flowmetry (Table 2). Three of them had a low erythrocyte count (about 1 per HPF), two had their erythrocyte volume histograms displaced to the left for unknown reasons and thus misinterpreted as glomerular bleeding, and three had high bacterial counts misinterpreted as small erythrocytes.

The Multistix 8 SG® dipsticks usually detect urine erythrocytes at counts above 5–20 per µl. However, significant haematuria has been defined as more than 11 erythrocytes per µl by flowmetry (i.e. more than about one erythrocyte per HPF) [21,25]. If the urine samples containing less than 11 erythrocytes per µl but with a 1+ positive dipstick for blood had been excluded, five cases with an incorrect diagnosis by flowmetry would have been avoided.

Urine microscopy correctly identified 28 of 31 patients with a glomerular disease and 38 of 48 patients with a urological disorder (P<0.0001), i.e. a sensitivity of 0.79 and a specificity of 0.90 for detecting non-glomerular bleeding. The positive and negative predictive values were 0.93 and 0.74 respectively.

According to these results, there was no significant difference between urine microscopy and flowmetry with respect to the discrimination between a nephrological and a urological source of bleeding.

**Discussion**

In the present study we have compared bright-field microscopy with automated urine flowmetry (Sysmex® UF-100) in their ability to discriminate between glomerular and non-glomerular haematuria. The study was designed to simulate a setting where patients were screened before assigning them to a urological or nephrological follow up. Automated urine flowmetry and clear-field microscopy by a skilled observer had the same sensitivity and specificity for identifying the source of haematuria. Thus, both methods should be of value when screening patients with haematuria. As automated urine flowmetry is less

---

### Table 1. Identification by flowmetry of glomerular or non-glomerular bleeding according to degree of haematuria in samples from 79 patients

<table>
<thead>
<tr>
<th>Dipstick test result for blood in urine</th>
<th>1+ Haematuria</th>
<th>2+ Haematuria</th>
<th>3+ Haematuria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of urine samples with haematuria (n)</td>
<td>n = 14</td>
<td>n = 32</td>
<td>n = 33</td>
</tr>
<tr>
<td>Number of urine samples with albuminuria (n)</td>
<td>n = 9</td>
<td>n = 21</td>
<td>n = 29</td>
</tr>
<tr>
<td>Urinary erythrocytes, as detected by flowmetry</td>
<td>7.3 ± 5.3 (1–17)</td>
<td>32.1 ± 33.6 (1–123)</td>
<td>899.5 ± 2604 (18–15000)</td>
</tr>
<tr>
<td>Mean ± SD (range); count per HPF (calculated)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urinary erythrocytes, as detected by microscopy</td>
<td>6.9 ± 7.5 (2–30)</td>
<td>16.1 ± 11.3 (3–50)</td>
<td>53.8 ± 35.9 (8–100)</td>
</tr>
<tr>
<td>Mean ± SD (range); count per HPF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Identification of glomerular or non glomerular urinary bleeding by urine (n)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C correct; U uncertain; I incorrect; HPF, high-power field (400 ×); SD, standard deviation.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 2. Results of urine flowmetry and corresponding pathological diagnosis in 79 patients with positive urine dipstick for blood

<table>
<thead>
<tr>
<th>Pathological diagnosis</th>
<th>Flowmetry pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glomerular</td>
</tr>
<tr>
<td>Urological disorders</td>
<td></td>
</tr>
<tr>
<td>Ureteric stones, prostatic trauma (TUR-p), and prostatitis</td>
<td>3</td>
</tr>
<tr>
<td>Prostatic cancer</td>
<td>1</td>
</tr>
<tr>
<td>Transitional-cell carcinoma</td>
<td>4</td>
</tr>
<tr>
<td>Renal-cell carcinoma</td>
<td>0</td>
</tr>
<tr>
<td>Total count (Urological disorders)</td>
<td>8</td>
</tr>
<tr>
<td>Nephrological diseases</td>
<td></td>
</tr>
<tr>
<td>IgA glomerulonephritis</td>
<td>12</td>
</tr>
<tr>
<td>Non-IgA glomerulonephritis</td>
<td>15</td>
</tr>
<tr>
<td>Subacute interstitial nephritis</td>
<td>0</td>
</tr>
<tr>
<td>Hypertensive and diabetic nephropathy</td>
<td>2</td>
</tr>
<tr>
<td>Total count (Nephrological diseases)</td>
<td>29</td>
</tr>
</tbody>
</table>

TUR-p, transurethral resection of the prostate. Glomerular and non-glomerular flowmetry patterns were identified from Kitasato criteria (major) and minor criteria as described above.
time-consuming than microscopy, the former may be the preferable procedure. Moreover, the presence of a physician is not necessary when the urine specimens are delivered to the laboratory, as the results are printed out in objective reports. The costs of analysis reagents are EUR 0.6 per urine sample (January 2001, Sysmex, Norway) which may be acceptable when the reduced manual workload in urine analysis is taken into account. Furthermore, it seems likely that an examination of the urine may be helpful when deciding where to begin—at either the urological or the nephrological clinic—thereby reducing the number of unnecessary investigations. On the other hand, we want to emphasize that urine flowmetry cannot replace urine microscopy when making clinical assessments of individual patients, but may reduce the number of microscopic examinations of the urines [21,23].

Automated flowmetry was quite reliable when identifying glomerular bleeding but was not so precise in identifying non-glomerular urinary bleeding. Unfortunately, this imprecision was seemingly but not significantly larger when examining bleeding due to transitional-cell carcinoma. Hyodo et al. [25] reported better results. However, they examined selected patients with haematuria of more than 2 erythrocytes per HPF—due mostly to IgA glomerulonephritis, transurethral resection of the prostate and extracorporeal shock-wave lithotripsy. The two latter conditions are associated with high erythrocyte counts. In our study, unselected patients with both low and high erythrocyte counts and with many different sources of urinary bleeding were included. Our data suggests that urine flowmetry is less reliable when urine samples contain less than 11 erythrocytes per µl (i.e. ≤1 erythrocyte per HPF). This may explain why we found a lower sensitivity and specificity with urine flowmetry. On the other hand, we introduced the minor criteria in addition to the Kitasato criteria, and this probably enhanced the present results.

Conclusions

Urine flowmetry had a sensitivity and specificity of 0.83 and 0.94 respectively in detecting non-glomerular bleeding among unselected patients with a positive dipstick for blood. The results of urine microscopy by a skilled observer were not different from flowmetry in this respect. Thus, automated flowmetry can be used in the differentiation between glomerular and non-glomerular haematuria. Furthermore, flowmetry is less time-consuming and provides more objective results than microscopy. Therefore, urine flowmetry may be cost effective in the management of patients with haematuria. Although urine flowmetry is fast and reliable, it cannot provide all of the detailed information obtainable by microscopic examination and consequently cannot replace urine microscopy when making clinical assessments of individual patients.

Acknowledgements. We wish to thank Ulrike Blaesio (Sysmex GMBH, Hamburg, Germany) and Benno Driese for their technical assistance, and Roald E. Strandjord for valuable comments on the manuscript.

References

8. de Caestecker MP, Ballardie FW. Unexplained haematuria. BMJ 1990; 301(6762): 1171–1172

*Received for publication: 22.11.00
Accepted in revised form: 2.3.01*