Differentiation of Renal from Non-Renal Hematuria by Microscopic Examination of Erythrocytes in Urine

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Recent studies indicate that hematuria of renal parenchymal origin can be differentiated from hematuria of other origin by the presence of dysmorphic urinary erythrocytes (cells exhibiting irregular membranes or small surface blebs). We investigated the utility of this simple screening assay in a routine clinical laboratory. Dysmorphic erythrocytes in urine from 69 patients (18 with renal-parenchymal disease) were quantified on unstained slides by medical technologists using phase-contrast microscopes. Samples stored at 4 °C or 23 °C for up to 5 h had no significant changes in percentages of dysmorphic erythrocytes (PDE). PDE was also not modified by urea nitrogen concentration, osmolality, or pH over the physiological ranges of these variables. Receiver-operating characteristic (ROC) curves indicated an optimal sensitivity of 88% and specificity of 94% at a decision level of 14% dysmorphic erythrocytes per high-power field. Thus, the presence of fewer than 14% dysmorphic cells is suggestive of extra-renal disease; more than 14% is suggestive of intra-renal disease.

Additional Keyphrases: dysmorphic erythrocytes • renal parenchymal disease • urinalysis • screening • receiver-operating characteristic curves • cutoff value • phase-contrast microscopy

Hematuria may result from various renal, urologic, or systemic processes and requires the immediate initiation of diagnostic procedures to identify the location of hemorrhage. The ability to differentiate intra-renal (glomerular) bleeding from extra-renal (nonglomerular) bleeding can help in the initial choice of diagnostic tests and minimize the expense and discomfort to the patient. Generally, intra-renal bleeding is investigated by examining renal biopsy tissue, whereas an extensive urologic work-up, including cystoscopy and retrograde pyelography, may be warranted to investigate extra-renal causes of hematuria. The presence of erythrocytes in urine sediment, in association with proteinuria and erythrocytic casts, is assumed to be evidence for glomerular damage (1). However, glomerular disease can occur in the absence of proteinuria (2, 3), and the few erythrocytic casts that are present are easily disrupted during sample processing (4, 5). Erythrocytic casts are present in a matrix of Tamm–Horsfall protein, a mucoprotein secreted by the ascending limb of Henle’s loop (6, 7). Although evaluation of urinary cells for the presence of this coating may be a useful aid in the diagnosing of urinary tract disease (8), the procedure requires specialized equipment and training.

Distinguishing an intra-renal from an extra-renal site of bleeding reportedly can be aided by microscopic examination of erythrocyte morphology in urine sediment (2, 10–14): erythrocytes from extra-renal sites appear normal, whereas those originating from renal parenchyma have dysmorphic characteristics (surface blebs or ruptured membranes). This approach was said to be valuable in screening patients with both micro and macroscopic hematuria (10). However, this technique has been used only by nephrologists examining urine specimens under specialized conditions (e.g., freshly voided urine examined under high-power microscopes).

Here we report our efforts to determine the applicability of this screening assay to the routine clinical laboratory. Urine samples from patients with hematuria were examined by medical technologists using phase-contrast microscopes. They counted the number of dysmorphic erythrocytes and correlated the findings with the diagnoses listed on the patients’ medical records. We also examined whether alterations of urea concentration, osmolality, pH, or time of examination after sample collection affected the number of erythrocytes noted.

Methods and Material

Patient Population

We studied 86 midstream urine samples from 69 patients [864% men, mean age 52 (SD 11) y] with hematuria, defined as four or more erythrocytes present per high-power field. Retrospective medical-chart review disclosed various diagnoses (see Figure 4 below). Diagnosis of glomerulonephritis and other renal parenchymal disease was based on biopsy. These diagnoses were not known to the medical technologists who evaluated the urine specimens.

Samples and General Protocol

We centrifuged 10 mL of urine at 1500 × g for 5 min in a 15-mL conical plastic centrifuge tube (Falcon, Oxnard, CA 93030) within 30 min of voiding. Nine milliliters of the resulting supernate was discarded and the sediment was resuspended in the remaining 1 mL.

A drop of this suspension was placed on a microscopic slide, covered with a coverslip (Type II, 33 mm square, size 2, thickness no. 1; Propper Manufacturing Co., Inc., Long Island City, NY 11103), and examined by phase-contrast microscopy under high power (400×). We used a phase-contrast module loaded on a Labophot microscope stand (Nikon, Tokyo, Japan 03-214-5311).

The medical technologists who examined the cells were trained in routine microscopic urinalysis procedures. To assess agreement between technologists, one technologist examined all of the samples; her results were then compared with results obtained by at least one other technologist. The observations of the other technologists were made independently of each other and within 15 min of the reference technologist.

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Guidelines for categorizing erythrocytes and a manual of photomicrographs exhibiting normal, ghost, and dysmorphic erythrocytes (e.g., Figure 1) were prepared in consultation with a staff nephrologist and were available at the bench for review. Dysmorphic cells exhibited irregular membranes or small surface blebs; normal cells were relatively uniform in size and shape and exhibited intact membranes. The technologists counted 100 cells per urine, categorizing them as either normal, dysmorphic, or ghost cells; crenated erythrocytes were scored as "normal."

Aliquots of the unprocessed urines were removed for various other examinations, including pH by dipstick (Lab-stix; Ames Division, Miles Laboratories, Inc., Elkhart, IN 46515), osmolality by freezing-point depression (Model 3DI; Advanced Instruments, Inc., Needham Heights, MA 02194), and urea nitrogen by an enzymatic conductivity electrode method (Astra-8; Beckman Instruments, Inc., Brea, CA 92621).

Time Study

A 10-mL urine sample was processed and analyzed without delay as described above ("time 0" samples). The remainder of the unprocessed urine was promptly divided into two aliquots. One was refrigerated at 4°C until use, the other left at room temperature. Portions of each sample were then processed and examined 1, 2, 3, 4, or 5 h later. All paired samples were examined within 10 min of each other by the reference technologist.

Alteration of Urinary Constituents

Samples of urine were processed and examined as described above (time 0 samples), with the remainder of the unprocessed urine being divided into various aliquots. To some aliquots we added either 0.1 volume of 8.0 mol/L NaCl solution (to increase osmolality), or 0.1 volume of 4.0 mol/L urea reagent. To study the effect of urine pH, we adjusted the pH of unprocessed aliquots to 5.0, 7.0, or 9.0 with either HCl or NaOH (0.2 mol/L each); no more than 1/20 volume of acid or base was needed to reach the appropriate values. All aliquots were then processed and examined by the reference technologist 1, 2, 3, or 4 h after the various additions. At least three different urine samples were examined at each time point to check for effects of pH, osmolality, or urea nitrogen.

Statistics

To assess agreement between technologists in quantifying dysmorphic erythrocytes, we compared the results obtained by two technologists for each of 66 specimens, calculating the discrepancy (d) between the technologists' values for each specimen by subtraction. From statistical theory (15), we know that, in the absence of any real technologist-to-technologist variation, the largest values of d are to be expected for specimens with approximately 50% dysmorphic cells; moreover, the values of d can be expected to be smaller as this percentage approaches 0% or 100%. Accordingly, we normalized the discrepancy values by converting them to z-scores as follows:

\[ z = \frac{|d|}{SE(d)} \]

where \( d = P_1 - P_2 \) = discrepancy between the proportion of dysmorphic cells observed by technologist 1 (\( P_1 \)) and the proportion of dysmorphic cells observed by technologist 2 (\( P_2 \)); \( SE(d) \) = standard error of \( d \) = \( \sqrt{\frac{(2P(1-P)}{n})} \); and \( P = (P_1 + P_2)/2 \).

To calculate receiver-operator characteristic curves (ROC analysis) (16), we used a computer program developed by Dr. Bruce Psaty, VA Medical Center, Seattle, WA, and run on an IBM PC. We used Student's t-test for paired data to assess the variations in quantifying erythrocytes at all times investigated; the Wilcoxon signed rank test and correlation coefficients were calculated with an IBM-PC computer with the use of a Microstat software package developed by Ecosoft, Inc., Indianapolis, IN 46220.

Results

Time study. Figure 2 illustrates the effects of temperature and time on the quantification of dysmorphic erythrocytes in urine. We saw no significant differences in the percentages of dysmorphic erythrocytes (mean 15.0, SD 6.2%) in urine stored at either room temperature or 4°C for 5 h, but after 24 h the numbers had significantly increased (\( P < 0.001 \)).

Effect of other urinary constituents. To investigate the pathogenesis of the changes in erythrocyte morphology, we measured the effects of osmolality, pH, and urea nitrogen concentration of urine samples and found no significant correlations between any of these variables and the percentage of dysmorphic erythrocytes. For urea nitrogen concen-

![Fig. 1. Examples of normal and dysmorphic erythrocytes as seen by phase-contrast microscopy: A) normal cell; B) ghost cell; C) dysmorphic cell extruding small bleb from membrane; D) dysmorphic cell with ruptured membrane (all 400×).](image-url)

![Fig. 2. Effect of time and storage temperature on the percentage of dysmorphic erythrocytes](image-url)
trations of 1050 to 14,850 mg/L in urine, the correlation coefficient \( r \) was 0.04; for osmolalities of 163 to 1290 mOsm/kg, \( r \) was 0.14; and for pH ranging from 5.0 to 8.5, \( r \) was 0.13.

When we modified the urines' pH, osmolality, and concentrations of urea nitrogen, any changes in the numbers of dysmorphic erythrocytes were not significantly different from the percentage found at time 0, and were within the range of inter-technologist variations in scoring.

**Technologist variation.** Figure 3 shows the amount of error predicted and observed when different technologists scored dysmorphic erythrocytes. This figure compares the cumulative distribution of observed discrepancies between paired examinations of the same slide by two technologists with the theoretically predicted distribution of these discrepancies in the absence of any technologist-to-technologist variation. The solid line shows the predicted distribution of discrepancies, as obtained from the normal or gaussian distribution—i.e., the distribution of discrepancies expected if the same technologist had performed both readings on all specimens. For example, the normal distribution predicts that about 67% of within-technologist discrepancies should yield a z-score of 1.0 or less.

The two lines are closely parallel, suggesting little technologist-to-technologist variation. We also used the Wilcoxon signed rank test to assess the variation between technologists. The test yielded \( P > 0.26 \) (z = 0.62), which indicated no significant differences between technologists in scoring dysmorphic erythrocytes.

**ROC analysis.** After retrospective medical-chart review we classified patients as having renal parenchymal diseases (glomerulonephritis, interstitial nephritis, tubular necrosis, etc.) or having hematuria of other etiologies (bladder infection, etc.), then correlated the patients' diagnoses (presence or absence of renal parenchymal disease) with the percentage of dysmorphic erythrocytes and ghost cells found in their urine sediment. As Figure 4 illustrates, in patients with renal parenchymal disease the proportion of dysmorphic cells was 19% or higher, whereas in patients with non-parenchymal disease it was 11% or lower.

Figure 5 shows ROC curves resulting from computer analysis of these data. In one of the ROC curves shown, ghost cells were included in the count of dysmorphic eryth-

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**Fig. 3.** Between-technologist variation in quantifying dysmorphic erythrocytes
The solid line represents the cumulative percent of samples as a function of z-score (generated from a z-score table), (●), between-technologist cumulative percent of samples as a function of z-score.

**Fig. 4.** Correlation of urine erythrocyte morphology with patients' diagnoses
The percentages of dysmorphic erythrocytes (dark bars) and ghost cells (open bars) and their 95% confidence intervals are shown for the various diagnostic groups in this study. The number of patients in each diagnostic group is indicated at the top of each pair of bars.

**Fig. 5.** Receiver-operating characteristic curves for quantification of dysmorphic erythrocytes
The percentages of dysmorphic erythrocytes—including (●) and excluding (△) ghost cells—were correlated with evidence of renal parenchymal disease as indicated in the patients' medical histories. Specificities and sensitivities were calculated from an ROC program; "A" denotes the point where they were maximized.
erythrocytes; in the other, ghost cells were excluded. A sensitivity of 82% and a specificity of 92% was obtained at a decision level of 21% for the first curve. But if ghost cells were scored as normal cells, a decision level of 14% gave the greatest discrimination between intra- and extra-renal groups, with a sensitivity and specificity of 88% and 94%, respectively. This latter decision level was chosen because it minimizes the number of false positives and false negatives. Thus, when fewer than 14% of the erythrocytes in urine are dysmorphic, the source of hematuria is probably extra-renal, whereas more than 14% dysmorphic cells is consistent with an intra-renal cause of hematuria.

Discussion

For patients with hematuria, it may be hard to localize the site of hemorrhage. Erythrocytic casts are considered evidence of glomerular bleeding, but their number may be small (2, 9) and they are easily disrupted by alkaline pH (5), urea-splitting bacteria, and centrifugation (4). Proteinuria is another, but insensitive, marker of glomerular damage.

Phase-contrast microscopic examination of urine erythrocyte morphology has been suggested as an easy, cost-effective, and non-invasive test to differentiate intra-renal from extra-renal hematuria (17, 18). We wanted to determine if this method could be used as a routine clinical laboratory procedure. We found that counts of dysmorphic erythrocytes obtained up to 5 h after voiding did not differ significantly from those obtained immediately after sample collection (Figure 2), but the numbers of dysmorphic cells had increased substantially by 24 h. We also found that the percentage of dysmorphic erythrocytes was not modified by pH, osmolality, or urea nitrogen concentration over the physiological ranges of these variables. The factors responsible for changes in urinary erythrocyte morphology are not known. Perhaps disruption of the cell membrane during passage through the glomerular basement membrane or the release of inflammatory products from neutrophils mediates the morphological changes (20).

From retrospective chart review, we determined whether patients had bleeding of intra-renal origin or extra-renal origin. This information and the experimental data on erythrocyte morphology were subjected to computerized receiver-operator characteristic (ROC) analysis.

The maximum sensitivity and specificity was obtained at a decision level of 21% dysmorphic cells (counting ghost cells as dysmorphic), or 14% (if ghost cells were excluded). We chose to use the latter cutoff values. This increases the specificity slightly and is consistent with the findings of Fairley and Birch (10) that these cells are often found in non-glomerular hematuria. The sensitivity of the test (88%) is comparable to that found in previous studies (2, 11, 17). Use of this decision level results in a minimum number of false-positives and false-negatives. Decision levels may be chosen by other criteria (e.g., so as to minimize only the number of false positives). We selected a cutoff that maximizes the overall performance of the screening test. Information obtained from this screen should be considered along with other patient data when one is selecting the most appropriate follow-up test.

Our decision level of 14% differs from that reported in other studies, where the cutoff was 80% dysmorphic cells (2, 10, 17). In these latter studies more-sophisticated microscopic equipment was used, set at higher magnification (1600×), or stained dry mounts were used in the examination. In this study, we used very rigorous criteria for classification of dysmorphic cells (evidence of extruded blebs from the membrane or a rupture in the membrane). In addition, the highest magnification employed was 400×, so the absolute number of dysmorphic erythrocytes counted is expected to be lower. Thus, our cutoff value is conservative. It is important that each laboratory establish its own decision level.

Interestingly, two patients with renal artery stenosis and hematuria did not display increased percentage of dysmorphic erythrocytes by these criteria. If these patients develop ischemic renal disease, we would expect to find a higher percentage of dysmorphic cells (considered intra-renal hematuria). If ischemic injury does not occur, a lower percentage of dysmorphic cells may be observed. Further studies are needed to clarify this point.

The results of the z-score analysis outlined in Figure 3 show that the magnitude of the variation in scoring cells between technologists was small, with a distribution similar to that which would be expected if one technologist were to make repeated readings on a specimen. The signed rank test shows that these discrepancies were random errors and not the result of one person consistently over- or under-scoring the number of dysmorphic cells. Evidently the test can be performed reliably in the clinical laboratory.

Thus, quantification of dysmorphic urinary erythrocytes by phase-contrast microscopy is a simple, cost-effective test that can aid clinicians in the identification of the site of hematuria. Erythrocyte morphology can be scored quickly, so the test is easily incorporated as a part of the standard microscopic urinalysis procedure.

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References


We would be happy to make copies of the "training set" available to interested readers. It may be useful to have some other references available as well. One we have found useful is "Urinary Sediment and Urinalysis", by Thomas Siamey, M.D., and Robert Kindrachuk, M.D., 1985, Saunders Co.


