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Dysmorphism of urinary red blood cells—Value in diagnosis

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Dysmorphism of urinary red blood cells—Value in diagnosis. To aid investigation into the clinical problem of hematuria, assessment of abnormalities in the shape of red cells in the urine (dysmorphism) is gaining popularity in nephrology. However, there is uncertainty in the literature regarding both the number of red blood cells (RBC) in normal urine, as well as the quantification of dysmorphism. We have shown that in normal urine ($N = 27$) the number of RBC is less than 2,000/ml as assessed by scanning electron microscopy of filtered urine specimens from normal volunteers without known renal disease, which compared to less than 1,000/ml by centrifugation and phase contrast microscopy of the same specimen. To determine whether dysmorphism of urinary red blood cells was a significant predictor of glomerular disease we compared the number of dysmorphic cells in the urine of patients with biopsy proven glomerulonephritis (GN), before and immediately after renal biopsy. We also compared the number of dysmorphic cells in patients with glomerulonephritis to those with lower urinary tract bleeding. Renal biopsy caused significant dysmorhpic hematuria, indicating that dysmorphism suggests renal rather than glomerular bleeding. Although patients with GN had significantly more dysmorphic urinary RBC when compared to those with lower tract urinary bleeding, the overlap was such that one could only be confident of renal hematuria if they accounted for greater than 75% of the total number of RBC. Non renal hematuria is present if number of dysmorphic cells is less than 17% of total RBC. Thus dysmorphism of urinary RBC is a useful diagnostic tool, but only if strict criteria established for each laboratory are adhered to.

There exists in the literature widespread disagreement and conflicting criteria regarding the level of hematuria which becomes pathologically significant and thus warrants investigation, especially in relation to the value of dysmorphism of urinary red blood cells as a guide to the etiology of hematuria. For instance, Birch and Fairley [1] quoted less than 2,000 red blood cells (RBC)/ml, Fairley and Birch [2] and Birch et al [3] less than 8,000 dysmorphic RBC/ml, and Fassett, Horgan and Matthew [4] less than 3,000 RBC/ml as normal, while Ihle, Long and Oats [5] considered less than 8,000 RBC/ml as normal but only performed a renal biopsy if the number of cells exceeded 20,000 RBC/ml, while still others only looked for dysmorphism itself [6] without regard to the number of red cells. Similarly, there is uncertainty as to how much dysmorphism can be considered normal and how various investigators have arrived at their criteria. There are several reports [3, 4, 7] which quote a figure of greater than 80% dysmorphic cells as indicating glomerular bleeding, but give no details as to how this figure was derived. Conversely, others regard greater than 10% to 14% of dysmorphic RBC as indicating glomerular pathology [8, 9], and others find mixed RBC morphology in glomerular, renal and lower urinary tract bleeding [10]. Since this technique is becoming increasingly used in nephrology and the problems outlined obviously cast serious doubt on the scientific basis underlying it which could lead to serious omissions of appropriate investigations, the present study was undertaken with the following aims:

1. To determine the number of red blood cells (RBC) in the urine of healthy volunteers by a centrifugation-independent method, and compare the values thus obtained with those using routine laboratory phase contrast microscopy on the same urine samples.
2. To compare the urine from patients with biopsy proven glomerulonephritis and those with proven lower urinary tract bleeding with respect to the content of dysmorphic RBC (DRBC), and define the limits of dysmorphism in these two conditions.
3. To determine the type of cell excreted in the hematuria caused by renal biopsy.
4. To establish the routine predictive value of RBC in the urine of patients undergoing routine renal function testing.

Methods

Red cell counting

To determine the total number of all RBC in the urine of volunteers without a known history of renal disease and independent of centrifugation, urinary red cells of all shapes and sizes were counted by scanning electron microscopy (SEM) of a filtered specimen and compared to a routine phase contrast microscopy (PCM) method. The total number of RBC in the urine of 27 volunteers was determined by phase contrast microscopy using 10 ml of a well stirred, early morning specimen of urine centrifuged at 2,000 rpm for four minutes; 9.5 ml of supernatant was discarded and the remainder resuspended and counted in a Fuchs Rosenthal counting chamber, as published previously [11]. Briefly, the entire field of the chamber (16 large squares, 0.0032 ml) is searched for casts and (16 small square, 0.0002 ml) are searched for red blood cells. A further 40

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ml of the same urine had 25% glutaraldehyde added to it, and half of this was used to determine normal urinary RBC by SEM.

For SEM, the samples were filtered through a 25 mm diameter filter paper with 2 μ pore size (Millipore Corp., Bedford, Massachusetts, USA) using a syringe. The filter papers were then dehydrated by slowly passing them through a graded series of ethanol concentrations (30, 50, 70, 90% and absolute) and then allowed to dry in air. Five 200 mesh grids were placed over the filter paper, and this was then sputter coated to give a 20 nm gold film. The number of recognizable RBC contained in 20 random squares, four from each grid of the filter paper, were counted in a J.E.O.L. SEM (JEOL, Tokyo, Japan) at 1,500 magnification and converted to RBC/ml. All recognizable RBC, irrespective of shape were counted and in doubtful cases magnification was increased. This method of counting cells on a filter paper was validated by counting a known number of red cells (500, 1,000 and 5,000 RBC/ml, $N = 18$). These were obtained from a stock solution of blood from a normal volunteer in which the total number of RBC were determined in a Coulter counter, in duplicate and from hematocrit measurements. Packed cells were then obtained from this by centrifugation, and the stock solution made up in 25% glutaraldehyde to contain 5×10^7 RBC/ml in a final glutaraldehyde concentration of 2.5%. Further dilutions were then made, immediately before use as desired, in 0.9% saline.

For each urine sample of a normal volunteer, the method was further checked by performing recovery assays on the urines by adding a known number of RBC to another portion of the urine. The dilutions and number of RBC in the stock solutions were checked each run by also counting the dilutions themselves.

Patient material

Thirty-one consecutive patients undergoing percutaneous renal biopsy for indications as determined by their specialists, had a first morning specimen of urine examined by phase contrast microscopy on the day of biopsy and also on the morning following the biopsy. Urine was tested for pH and blood with an analytical reagent strip.

If microscopy was not undertaken immediately the urine was fixed with 0.5 ml 16% formalin for later counting, which occurred in 14 specimens following biopsy. The total number of RBC, and numbers of normal and dysmorphic RBC were counted. Cells were regarded as dysmorphic if they exhibited a wide range of morphological variation frequently with loss of hemoglobin, and normal if not more than two cell populations were present, either well hemoglobinized with a relatively normal appearance or showed loss of hemoglobin or other minor changes, according to the criteria of Birch et al [3]. A minimum of 20 RBC were counted each time to ensure an adequate representation of RBC morphology. Assuming that the increase in urinary RBC excretion following renal biopsy was traumatic in origin and thus normomorphic in nature, the expected fraction of normomorphic cells past biopsy was calculated by the following equation:

$$\frac{\text{Normomorphic cells pre-biopsy} + \text{Increase in total RBC}}{\text{Total RBC post-biopsy}}$$

and expressed as a percentage.

A further 28 patients with proven lower urinary tract bleeding (renal calculi and bladder tumours) also had their first morning urine specimen examined immediately for the number of normal and dysmorphic cells by phase contrast microscopy. These patients had normal renal function as assessed by a normal creatinine clearance, no casts [12] evident in an early morning specimen of urine and no history of renal disease. Although renal biopsy could not ethically be performed on these patients to definitively exclude coincident renal parenchymal disease, it was assumed the etiology of hematuria in these patients was due to lower urinary tract bleeding.

To assess the practicality of routine urine examination for dysmorphic cells, 64 consecutive patients referred for renal function testing had their early morning urine examined by PCM for dysmorphic cells as outlined above.

The presumptive diagnoses of these patients and the correlation with urine microscopy were only established when all the 64 results were available. The patients were considered to have glomerular disease if they either had [1] a history of, and/or biopsy proven glomerulonephritis *and* had no history of kidney stone disease, pyelonephritis, reflux nephropathy, or analgesic abuse, or [2], in the absence of any positive history, if the renal function tests [12] showed an abnormal GFR only, without a specific concentrating or acidification defect (independent of GFR). They were considered to have medullary disease if [1] they did not have either a history and/or biopsy proof of glomerulonephritis and had a history of any of the following: kidney stone disease, pyelonephritis, reflux nephropathy or analgesic abuse in the presence of a concentrating or acidification defect, or [2] in the absence of any positive history, if they either had a specific concentrating defect (that is, independent of GFR abnormalities) and/or an acidification defect. Seventeen patients did not fit clearly into either of the two categories.

Phase-contrast urine microscopy was performed by two observers who were blinded to the final diagnosis. Uniformity in the interpretation of RBC morphology was achieved by dual examination of 21 urine specimens which demonstrated close agreement in recognition of dysmorphic cells ($r = 0.85$). In eleven of these cases initial examination was performed on freshly voided urine and subsequent examination was done after fixation in 0.5 ml of 16% formalin. Similar agreement in recognition of dysmorphic cells was seen ($r = 0.89$) indicating that fixation had no effect on RBC morphology. Histological lesions were regarded as mild if light microscopy revealed minimal abnormality, the majority of pathology demonstrated on immunofluorescence or electron microscopy and creatinine clearance was greater than 80 ml/min, moderate if light microscopic (± immunofluorescence and electron microscopy abnormalities) were evident in association with a creatinine clearance of greater than 60 mls/min and severe if proliferative or inflammatory lesions were evident in association with a creatinine clearance of less than 50 ml/min.

Statistical analysis of patients undergoing renal biopsy was made using a paired *t*-test. Other analyses were made using a two-sample *t*-test

For the purposes of comparison unless otherwise stated, crenated cells were regarded as dysmorphic, since according to Birch et al [3] these cells exhibited a wide range of morphological variation and thus fulfilled the criteria for dysmorphism.

Table 1. Validation of scanning electron microscopic method of counting red blood cells and comparison of number of red blood cells in normal human urine by phase contrast and scanning electron microscopy

Validation of SEM in counting RBC		Normal urinary RBC in counting comparison of PCM & SEM	
Known no. of cells	Scanning electron microscopy	Phase contrast microscopy	Scanning electron microscopy
500	554 ± 58 (5)	203 ± 72 (27)	526 ± 142
1000	1054 ± 99 (8)	(949)[a]	(2206)[a]
5000	5194 ± 264 (5)	(0–1750)[b]	(94–3570)[b]
Correlation			
r	0.982	0.814	
P	0.001	0.001	

Data presented are means ± SEM. Number in parentheses are the number of samples.
[a] Upper 95% level
[b] Actual range

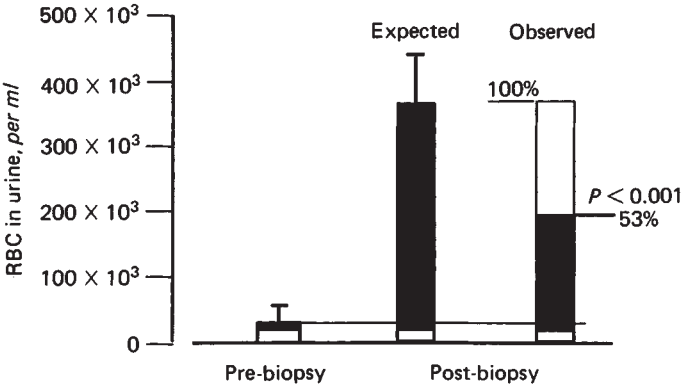

Fig. 1. *Hematuria before and following renal biopsy normomorphic cells indicated by solid bar, dysmorphic by open bar.* Also indicated is the expected number of normomorphic cells post-biopsy (100%) versus number actually observed (53%, $P < 0.001$).

Table 2. Comparison of hematuria pre- and post-biopsy in patients with glomerulonephritis and in patients with lower urinary tract bleeding

	Pre biopsy (26)	Post biopsy (26)	Lower tract bleeding (28)
Total RBC/ml	29123 ± 11038	331327 ± 226251	98446 ± 23642
Dysmorphic RBC %	65.7 ± 4.8	62.6 ± 5.6	48.5 ± 2.5

Data presented are means ± SEM.

Results

The SEM method of counting red cells of a filtered specimen was well validated with a correlation coefficient of 0.98 (Table 1) using a known number of RBC in saline. Similarly, the recovery rate of a known number of RBC in urine was 97%. Both indicate a high degree of reliability of the technique.

Determination of the number of RBC in normal volunteers' urine is summarized in Table 1, confirming that normal urine contains <1,000 RBC/ml as assessed by PCM (95% confidence). SEM showed higher RBC excretion rates, as expected, with a mean of 726 RBC/ml (2,206 RBC/ml, 95% upper limit).

Of the 31 patients who underwent renal biopsy, 26 patients increased their urinary rbc excretion following biopsy by 303,203 ± 116,818 (mean ± SE). The remaining five patients had fewer urinary RBC/ml following biopsy. Since the aim of the study was to determine the type of red cell excreted due to the biopsy, these five patients were not considered further. Table 2 demonstrates that there was no difference in the percentage of dysmorphic RBC seen in the urine following biopsy compared with pre-biopsy. This was true whether crenated cells were regarded as dysmorphic or normomorphic, and as their morphology varied widely and criteria for dysmorphism is unclear, they were subsequently regarded as dysmorphic (57.4 ± 5.4%, 56.0 ± 6.1 and 31.6 ± 2.1% without crenated cells respectively, Table 2). If the increase in total RBC excretion seen after renal biopsy was all "non-glomerular" in nature, 84.4% ± 3.76 of total urinary RBC following renal biopsy (**Methods**) would be expected to be of normal morphology as opposed to the observed 37.3 ± 5.2% (53% of the increase in RBC, rather than 100% of the increase, Fig. 1). This difference between observed and expected percentages of normal RBC was statistically highly significant ($P < 0.0001$). Histological diagnosis included IgA nephropathy −8, mesangial proliferative glomerulonephritis (IgA negative) −2, membranous glomerulonephritis −4, focal sclerosing glomerulonephritis −2, focal necrotizing glomerulonephritis −1, transplant rejection −2, post-infectious glomerulonephritis −1, afferent arteriolar C3 deposition or thin basement membrane disease −11. Histological severity of the glomerulonephritis was graded as severe ($N = 7$), moderate ($N = 13$), or mild ($N = 11$), and there was no difference in degree of urinary RBC dysmorphism among the three groups being 72% ± 15, 55% ± 22 and 78% ± 22 and 78% ± 25, respectively.

The results of the 28 patients with lower urinary tract bleeding are presented in Table 2. They had significantly lower percentage of dysmorphic cells when compared with the patients with biopsy proven glomerulonephritis (49% vs. 66%, $P < 0.005$). However, the range of values obtained were such that when considering the 95% confidence limits, considerable overlap occurred, as illustrated in Figure 2. Some patients with histologically-proven glomerular pathology had only 17% dysmorphic cells, whereas some patients with lower urinary tract bleeding had up to 75% dysmorphic cells.

If up to 8,000 dysmorphic RBC are regarded as normal, as defined by Birch et al [3], 10 patients with glomerulonephritis and 26 patients with lower tract hematuria had abnormal numbers of dysmorphic red cells in their urine. Of the 16 patients with glomerulonephritis with less than 8,000 dysmorphic RBC, 15 patients had >2,000 normal RBC, and one patient had 7,000 RBC of which 6,750 were dysmorphic. When the patients were compared according to these criteria with 8,000 dysmorphic RBC excluded from the initial number of dysmorphic cells and then recalculated as a percentage, there was no significant difference between the dysmorphic RBC in the urine of patients with glomerulonephritis (58.1 ± 33.2 SD) or lower urinary tract bleeding (41.0 ± 19.1% SD, $P > 0.05$).

The results of the 64 patients undergoing renal function testing with respect to morphology of RBC is shown in Table 3. The percentage of dysmorphic cells in patients with glomerular,

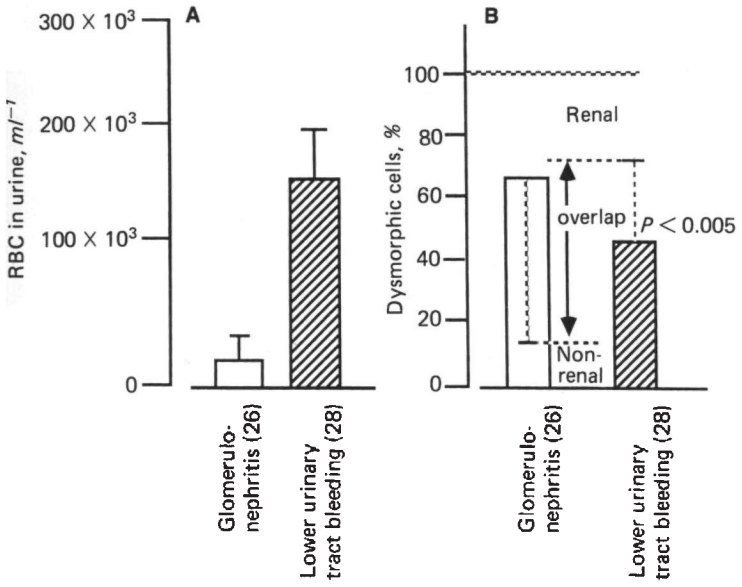

Fig. 2. *Degree of hematuria (A) and percent dysmorphism (B) in patients with proven glomerulonephritis (□) and with lower tract bleeding (▨).* The latter had significantly lower percentage dysmorphism than patients with glomerulonephritis. The overlap in dysmorphic cells between the groups (±2 SD) demonstrates that some patients with lower urinary tract bleeding had up to 75% dysmorphic cells, and some patients with glomerular bleeding had as few as 17% dysmorphic cells.

Table 3. Comparison of number of red blood cells and degree of dysmorphism in 64 patients undergoing one-day renal function testing with significant hematuria (>1000 RBC/ml)

	Total number of RBC (per ml)	Percent dysmorphic
Glomerular (16)	32 ± 9.4 × 10^3	67 ± 4.9
No. of patients with >75% dysmorphic cells	6/16	
Medullary (25)	13656 ± 4.5 × 10^3	63 ± 4.2
No. of patients with >17% of dysmorphic cells	0/25	
Uncertain (17)	160 ± 138 × 10^3	75 ± 3.7
No hematuria (6)	708 ± 70	66 ± 5.5

Number in parentheses are number of patients with significant hematuria (>1,000 RBC/ml).

Data are presented as mean ± SEM.

medullary or uncertain etiology, as previously defined (**Methods**), was not significantly different.

The number of crenated RBC, but not those of dysmorphic cells, increased significantly with increasing pH ($r = 0.41$, $P < 0.04$). This strongly implies that the mechanisms responsible for producing dysmorphism are very different to those producing crenation. However, since previous workers have regarded crenated cells as dysmorphic, for reasons of comparison, in this paper we have also counted them as dysmorphic unless otherwise stated. In addition, as pointed out above, inclusion or exclusion of crenated cells in the analysis in no way altered the conclusions this study led us to.

Discussion

Hematuria is a common diagnostic problem in clinical practice. With the aid of sensitive dip-stick analysis of urine more patients are referred for investigation of microscopic hematuria. However, there remains controversy in the literature regarding the level at which hematuria is a cause of concern.

Birch and Fairley in their initial report of dysmorphism of urinary RBC as a guide to renal pathology regarded up to 2,000 RBC/ml as being normal [1], however, in subsequent reports they regard up to 8,000 dysmorphic RBC/ml as normal [2, 3]. Others have regarded greater than 3,000 RBC/ml as abnormal regardless of RBC morphology [4].

We have previously shown that the upper limit of normal urinary red cell excretion was up to 1,000/ml [11, 13], and the current report confirms this with the "gold standard" technique. By using SEM and a filtering technique, all urinary RBC are counted and a much higher degree of accuracy can be achieved. The higher values obtained with SEM is not surprising as not all cells could be expected to be sedimented by centrifugation. We, as others [4] cannot regard 8,000 RBC/ml as normal. The reason for this discrepancy is unclear as the sole difference in methodology is the examination of early morning as opposed to random urine samples, but it is difficult to accept that phase contrast microscopy would offer a better degree of resolution than scanning electron microscopy. On the contrary, we have great difficulty in being sure when a fragment, as seen in the PCM is indeed part of a cell or just unrecognizable sediment (crystals, etc.).

Dysmorphism of urinary rbc has been regarded as an indicator of glomerular pathology [3, 4, 7–9, 13, 14]. However, at what level dysmorphic RBC becomes significant is also unclear. Birch and Fairley [1, 2] regarded up to 8,000 dysmorphic cells as normal and accordingly exclude this amount when assessing urinary RBC with respect to morphology. They recommend investigation of the lower urinary tract in all patients with any normomorphic urinary RBC. Others assess the pathology to be glomerular if >80% RBC are dysmorphic and non-glomerular if >80% RBC are normomorphic [4, 7] and still others regard >10% dysmorphism to be indicative of glomerular bleeding [8]. Thus, many patients assessed by the latter criteria as having glomerular bleeding would have lower urinary tract bleeding when assessed by the criteria of Birch and Fairley [1, 2].

The present study indicates that while patients with glomerulonephritis have significantly larger percentages of dysmorphic RBC, it is only useful as an adjuvant in diagnosis if the

percentage exceeds >75%. At levels below this lower urinary tract bleeding remains a possibility.

Furthermore, the study demonstrates that dysmorphism of urinary RBC is a non-specific indicator of renal pathology and not indicative of purely glomerular disease as evidenced by the fact that patients undergoing renal biopsy had equivalent percentages of dysmorphic RBC both pre- and post-biopsy. Although the results from patients undergoing renal function testing are less rigorous, the fact that patients with glomerular or medullary pathology could not be separated according to the dysmorphism of urinary RBC, would underscore the need to be extremely cautious in labelling any patient's significant hematuria as of either glomerular or lower tract origin. Since dysmorphism is not indicative only of glomerular bleeding but of renal bleeding, patients with significant interstitial disease, such as due to analgesic nephropathy or connective tissue diseases might also demonstrate significant (by our criteria) dysmorphism of their urinary RBC.

We thus conclude that, by phase contrast microscopy, normal urine contains less than 1,000 RBC/ml and that dysmorphism of urinary RBC is a useful clinical predictor of renal pathology (not purely glomerular) as opposed to lower urinary tract bleeding, only if they account for greater than 75% of the total number of urinary RBC. Lower urinary tract bleeding, on the other hand, can only be diagnosed with certainty with this technique if there are more than 83% non-dysmorphic cells present. At levels between these two extremes the finding of dysmorphic RBC is non-specific in nature and investigation of hematuria should proceed according to other parameters such as history, presence of proteinuria, tubular defects and the presence or absence of casts in the first morning specimen of urine [11].

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