Evaluation of light microscopy to localise the site of haematuria

B Rath, C Turner, B Hartley, C Chantler

Abstract

Red cell morphology was examined by light microscopy in 122 urine specimens from 99 patients with haematuria in an attempt to define the site of origin. Altogether 84% of glomerular bleeding and 91% of non-glomerular bleeding was correctly assigned according to diagnoses determined by renal biopsy in 51 patients and clinically in 48. The test should be interpreted with caution as both false positive and false negative results were observed, some in the same individual with unchanged pathology at different times. No advantage of phase contrast over bright field microscopy was apparent even when examining uncentrifuged urines.

The observation of Birch and Fairley that red cell morphology could be used to localise the site of haematuria in the urinary tract^{1,2} has been confirmed by a number of other groups.^{3–6} All used phase contrast microscopy; this is, however, frequently unavailable and therefore limits the usefulness of this otherwise simple and inexpensive test. Another problem associated with the technique is high interobserver variation due mainly to confusion in distinguishing normal and abnormal cell types.⁷ Birch and Fairley classified crenated cells as normal and anisocytosis and bizarre deformed cells as diagnostic of glomerular bleeding. Fassett *et al* considered cells with surface projections (echinocytes) as abnormal,⁸ but Bessis and Mohandas have shown that echinocytes and stomatocytes are normal variants as they regain normal morphology after adjustment of pH and ionic strength.⁹ We have recently observed that low osmolality (<300 mmol/kg) can preferentially reduce deformed cell counts (unpublished observations). The morphological pattern may also vary in the same patient at different times.¹⁰

In the present study we have evaluated the use of routine bright field rather than phase contrast microscopy in prediction of the site of bleeding in the urinary tract, and further assessed the reliability of the test, using the morphological criteria of Birch and Fairley.

Methods

One hundred and twenty two spot mid-stream urine specimens, all at least 1+ blood on dipstick testing (BM Test 5L, Boehringer Mannheim) and with red cell count greater than $20 \times 10^6/l$, were collected from 99 patients. Diagnostic details are given in table 1. Multiple

samples (two to five specimens collected at least 24 hours apart—usually one week) were obtained from 16 of the patients in order to assess the within patient variability of the method. Altogether 100 samples were from patients with glomerular disease and 22 from patients with lower tract disease. Red cells were counted using a Neubauer chamber, and morphological studies performed 'blind' at $\times 450$ magnification using uncentrifuged specimens within one hour of collection. The first 20 specimens were examined with both phase contrast and bright field microscopes and no differences in interpretation were apparent. Subsequent work used bright field microscopy only. Eighty per cent dysmorphic cells was taken to indicate unequivocal glomerular bleeding, 20–80% dysmorphic cells as a mixed picture, and less than 20% as non-glomerular bleeding.^{2–4}

Casts were counted in the sediments from 10 ml aliquots of the urine specimens, centrifuged for five minutes at 540 g and resuspended in 0.2 ml supernatant urine. Only red cell, cellular, and granular casts were analysed. Proteinuria was assessed by dipstick testing (BM Test 5L test strips).

Results

The localisation of bleeding inferred from morphological studies of red cells is compared with conventional diagnosis in table 2.

An unequivocal glomerular pattern (>80% dysmorphic red cells) was found in 60/100 samples from patients with glomerular disease. A further 26 samples from patients with glomerulopathies showed a mixed picture. Fourteen specimens from 13 patients with glomerular disease, 10 proved at biopsy, had less than 20% of deformed red cells in their urine and would thus have been misclassified. Two of 22 samples from patients with lower tract bleeding had high proportions of deformed red cells, one greater than 80%, the other a mixed pattern with 71% deformed cells.

The effect of cell number on the reliability of the test is also shown in table 2. There was no difference in the diagnostic sensitivity between samples with $20\text{--}50 \text{ cells} \times 10^6/l$, $51\text{--}100 \text{ cells} \times 10^6/l$, or more than $100 \text{ cells} \times 10^6/l$ for the diagnosis of glomerular bleeding. However both the samples giving inappropriate patterns from lower tract bleeding had low cell counts (30 and $50 \text{ cells} \times 10^6/l$). In our series of samples the sensitivity of the technique, taking mixed pictures as diagnostic of glomerular bleeding, was 86–87% with a specificity of 91%. The predictive value of the test was 98% for glomerular

Evelina Children's Department,
United Medical and Dental Schools of
Guy's and St Thomas's Hospitals,
12th Floor Tower,
Guy's Hospital,
London Bridge,
London SE1 9RT
B Rath
C Turner
B Hartley
C Chantler

Correspondence to:
Dr Turner.

Accepted 1 November 1990

Table 1 Patients and diagnoses

Disease	Total No. patients	No of patients biopsied	No of clinical diagnoses	Total No samples
Glomerular				
Acute post-streptococcal glomerulonephritis	1	1		2
Alport's syndrome	19	5	14	22
Chronic glomerulonephritis	1		1	1
Diffuse mesangial sclerosis	1	1		1
Transplant rejection	1	2		2
Focal glomerulonephritis	1	1		1
Focal segmental glomerulosclerosis	1	1		4
Henoch-Schönlein purpura	9	6	3	13
Haemolytic uraemic syndrome	2		2	2
IgA nephropathy	10	10		13
Loin pain haematuria	1	1		1
Mesangiocapillary glomerulonephritis	5	5		8
Mesangial proliferative glomerulonephritis	2	2		2
Minimal change nephrotic syndrome	2		2	2
Membranous nephropathy	2	2		3
Polyarteritis nodosa	2	2		3
Reflux nephropathy	7	1	6	7
Sickle cell trait	1	1		1
Systemic lupus erythematosus	7	7		11
Thin membrane disease	1	1		1
Subtotal	77	49	28	100
Non-glomerular				
Adult polycystic kidney disease	3		3	3
Calculi	2		2	2
Bladder carcinoma	1	1		1
Interstitial nephritis	1	1		1
After renal biopsy	2		2	2
Postoperative	8		8	8
Trauma	1		1	1
Urinary tract infection	3		3	3
Vulvovaginitis	1		1	1
Subtotal	22	2	20	22

Table 2 Comparisons of diagnosis with urinary red cell morphology. Results are number (%)

Cells×10 ⁶ /l	No of samples	Microscopic picture		
		Glomerular	Mixed	Non-glomerular
Glomerular disease:				
21-50	16	9 (56)	4 (25)	3 (19)
51-100	13	7 (54)	5 (39)	1 (8)
>100	71	44 (62)	17 (24)	10 (14)
All samples	100	60 (60)	26 (26)	14 (14)
Non-glomerular disease:				
21-50	3	1 (33)	1 (33)	1 (33)
51-100	2			2 (100)
>100	17			17 (100)
All samples	22	1 (5)	1 (5)	20 (91)

bleeding and 59% for lower tract bleeding, rising to 100% and 63% respectively for cell counts greater than $50 \times 10^6/l$. Within patient variation is shown in table 3. All patients had glomerular disease. Five of 16 showed an unequivocal glomerular pattern on both occasions studied, and 2/16 were incorrectly assigned on both occasions. One of these latter patients had bacteriuria and other evidence of urinary tract infection which may account for the erroneous results. Three of 16 patients gave mixed pictures and lower tract pictures at different times and 2/16 a combination of all three pictures. We could discern no association between the pattern of haematuria and the course of the disease.

Casts (38 red cell, nine renal tubular cell, pus cell or coarse granular casts) were present in 47 samples, all from patients with glomerular disease. In 10 of these (nine with glomerulopathies proved at biopsy and one with Alport's syndrome) a mixed morphological picture had been observed; three had shown a lower tract pattern. There was a significant correlation

Table 3 Microscopic picture in multiple samples from individual patients

Diagnosis	Microscopic picture (% dysmorphic cells)
Henoch-Schönlein purpura	>80, >80, >80, 20-80, 20-80
Henoch-Schönlein purpura	>80, 20-80
Henoch-Schönlein purpura	>80, >80
Alport's syndrome	>80, >80
Alport's syndrome*	20-80, <20
Alport's syndrome	>80, >80
Mesangiocapillary glomerulonephritis*	>80, >80
Mesangiocapillary glomerulonephritis*	>80, >80, 20-80
Mesangiocapillary glomerulonephritis*	>80, 20-80, 20-80
Systemic lupus erythematosus*	<20, <20
Systemic lupus erythematosus*	>80, >80, 20-80, <20
IgA nephropathy*	>80, >80
Membranous nephropathy*	<20, <20
Polyarteritis nodosa*	20-80, <20
Focal segmental glomerulosclerosis*	>80, 20-80, <20
Acute post-streptococcal glomerulonephritis*	20-80, 20-80, <20

*Proved at biopsy.

between red cell number and casts ($r=0.56$, $p<0.001$).

Proteinuria did not correlate with red cell morphology in our study. Altogether 19/22 samples from patients with lower tract bleeding had 1+ or greater (>0.3 g/l) protein on dipstick testing and 24/100 samples from patients with glomerular haematuria were protein negative.

Discussion

It has been claimed that the site of bleeding in haematuria can be assessed with high sensitivity and specificity ($>95\%$) by analysis of red cell morphology using phase contrast microscopy.¹⁻⁶ Rizzoni has stated that the standard microscope is equally reliable.¹¹ We found no

difference at all between phase contrast and bright field microscopy, but found red cell morphology gave misleading results in 40% of glomerular (26% mixed picture, 14% non-glomerular type pattern) and 9% of non-glomerular cases. Urinary tract infection interfered with the interpretation of red cell morphology in at least one of our patients. It is possible that our use of uncentrifuged specimens affected the reliability of the test as a number of workers claiming higher sensitivities used centrifuged specimens. However, Venkatraman *et al* compared centrifuged and uncentrifuged specimens and found little difference.⁷

In our study 26/27 samples with mixed red cell patterns came from patients with glomerular bleeding, seven of these also had pathological casts. We therefore agree with Rizzoni *et al* that mixed pictures are predominantly glomerular.⁵ If these criteria are accepted the sensitivity of localisation of haematuria in the present study is 86% for glomerular (98% predictive value) and 91% for non-glomerular bleeding (63% predictive value). Our conclusions are therefore that the study of red cell morphology in the urine of patients with haematuria can be a useful guide to the site of bleeding, but should be combined with other tests as there are a number of patients in whose samples the pattern is inappropriate or equivocal. Very dilute samples or samples from patients with bacteriuria may be misleading. Mixed pictures imply glomerular origin. The degree of haema-

turia does not affect the result provided the red cell count is above $50 \text{ cells} \times 10^6/\text{l}$. Reliability is not compromised if uncentrifuged urine is inspected using bright field rather than phase contrast microscopy, making the test particularly simple, rapid, and inexpensive.

We thank the Children Nationwide Medical Research Fund for financial support. Dr Rath was supported by a Commonwealth Medical Fellowship.

- 1 Birch DF, Fairley KF. Haematuria: glomerular or non-glomerular? *Lancet* 1979;ii:845-6.
- 2 Fairley KF, Birch DF. Hematuria: a simple method for identifying glomerular bleeding. *Kidney Int* 1982;21:105-8.
- 3 Fassett RG, Horgan BA, Mathew TH. Detection of glomerular bleeding by phase-contrast microscopy *Lancet* 1982;i:1432-4.
- 4 Stapleton FB, Brown RE, Koh SJ. Dysmorphic erythrocytes in children with hematuria: characterisation, recognition and diagnostic application. *Pediatr Res* 1983;17:358A.
- 5 Rizzoni G, Braggion F, Zacchello G. Evaluation of glomerular and non-glomerular hematuria by phase-contrast microscopy. *J Pediatr* 1983;103:370-4.
- 6 De Santo NG, Nuzzi F, Capodicasa G, *et al*. Phase contrast microscopy of the urine sediment for the diagnosis of glomerular and non-glomerular bleeding—data in children and adults with normal creatinine clearance. *Nephron* 1987;45:35-9.
- 7 Venkatraman C, Pead L, Lee HA, Maskell R. A blind controlled trial of phase-contrast microscopy by 2 observers for evaluating the source of hematuria. *Nephron* 1986;44:304-8.
- 8 Fassett RG, Horgan B, Gove D, Mathew TH. Scanning electron microscopy of glomerular and non glomerular red blood cells. *Clin Nephrol* 1983;20:11-6.
- 9 Bessis M, Mohandas N. Red cell structure, shapes and deformability. *Br J Haematol* 1975;31 (suppl):5-10.
- 10 van Iseghem P, Hanglustaine D, Bollens W, Michielsens P. Urinary erythrocyte morphology in acute glomerulonephritis. *BMJ* 1983;287:1183.
- 11 Rizzoni G, Braggion F, Grando F, Baraldi E. Detection of glomerular and nonglomerular bleeding. *J Pediatr* 1984;104:161.

More on cytokines

I referred recently to the possible role of cytokines in meningitis.¹ Now workers in the Gambia (Kwiatkowski *et al*. *Lancet* 1990; 336: 1201-4) have studied plasma concentrations of cytokines in children with malaria. Concentrations of tumour necrosis factor on the first day of illness in children with non-cerebral malaria were about twice those of children with other illnesses. In those who had cerebral malaria but survived the increase was five-fold but in those who died of cerebral malaria concentrations were increased by a factor of 25 or more. With antimalarial treatment the concentrations fell in the survivors but not in those who eventually died. Similar but much less pronounced changes were seen in plasma concentrations of interleukin 1 alpha.

Whether the increase in circulating cytokines is simply a reflection of the severity of the disease process or part of the pathogenesis of cerebral malaria is as yet uncertain. Presumably, as in meningitis, the next step will be a trial of steroid or other drugs which inhibit cytokine release.

ARCHIVIST

1 Archivist. Dexamethasone in meningitis. *Arch Dis Child* 1991;66:137.