

The (fixed) urinary sediment, a simple and useful diagnostic tool in patients with haematuria

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ABSTRACT

Examination of the urinary sediment is a simple and indispensable tool in the diagnostic approach to patients with asymptomatic haematuria. Various glomerular and nonglomerular diseases can cause haematuria. A well-trained expert can distinguish between these two forms of haematuria by examining the urinary sediment under a simple light microscope. In glomerular haematuria, dysmorphic erythrocytes and erythrocyte casts are found, whereas in nonglomerular haematuria the erythrocytes are monomorphic and erythrocyte casts are absent. However, few people have sufficient expertise in the examination of the urinary sediment, and consequently this investigation is performed far too seldom. A few years ago, a simple method of fixation of the urinary sediment became available. Fixed specimens can be stored at room temperature for at least two weeks, which enables the sending of a fixed specimen to an expert examiner by regular mail. In this way, the urinary sediment can more frequently be used as the initial investigation in the diagnostic route of patients with asymptomatic haematuria.

INTRODUCTION

Persistent or intermittent haematuria is an alarming symptom for the patient and his doctor. Haematuria can be caused by a number of conditions, including infections and stone disease of the urinary tract, malignant disorders, coagulation disorders and intrinsic renal diseases (for example glomerulonephritis). The medical history and physical examination might provide clues that point

towards the diagnosis. On the other hand, determining an adequate diagnostic strategy will be more difficult when additional symptoms are missing. This asymptomatic haematuria can be macroscopic or microscopic, the latter being usually discovered coincidentally.

In the case of asymptomatic haematuria, most patients are sent for urological evaluation, most likely because of concern for a malignancy of the urogenital tract.¹ This urological evaluation usually consists of imaging of the urinary tract (ultrasound, intravenous urography, CT scan), blood and urine chemistry, urine cytology and cystoscopy.^{2,3} However, a urological evaluation is inappropriate when glomerular disease is present. In that case other diagnostic instruments, such as renal biopsy, will be necessary to disclose the nature of the disease. Since examination of the urinary sediment can help to differentiate between glomerular and nonglomerular forms of haematuria, it is an important tool in patients with asymptomatic haematuria. Birch and Fairley were the first to show that examination of the urinary sediment by phase-contrast microscopy can help in the discrimination between glomerular and nonglomerular forms of haematuria.^{4,5} Nowadays, examination of the urinary sediment is mostly used to screen for urinary tract infection or to confirm the presence of haematuria. Most prevailing diagnostic algorithms for the analysis of asymptomatic haematuria advise extensive urological evaluation,^{2,3,6-8} whereas only a few stress the role of the urinary sediment as a diagnostic instrument.^{8,9} In a recently published diagnostic algorithm, the urinary sediment was not mentioned at all.⁶ By following such a strategy, the majority of serious urological pathology will be disclosed. Unfortunately, in many studies a definite

urological diagnosis was made in only 30 to 60% of cases.^{1,10-13} Furthermore, many patients with nephrological diseases will remain unidentified, and a considerable number of patients will endure inappropriate and often repeated urological testing. We estimated on the basis of a retrospective study that about 25% of 134 patients referred to a urology department for the analysis of asymptomatic haematuria most likely had glomerular disease and thus underwent inappropriate urological testing [unpublished data]. Therefore, examination of the urinary sediment in patients with haematuria is mandatory, preferably before a patient is referred to a medical specialist. In this review we stress the importance of this simple, cheap and informative test as a diagnostic tool in patients with asymptomatic haematuria. Furthermore, we bring into notice a method of fixation of the urinary sediment that allows preservation of the urinary specimen for two weeks at room temperature.

PREVALENCE OF ASYMPTOMATIC HAEMATURIA

The prevalence of asymptomatic microscopic haematuria has been determined in unselected populations, as well as in screening studies of selected populations. Woolhandler and co-workers reviewed five population-based studies, and mention a prevalence of asymptomatic haematuria of 0.19 to 16.1%.¹⁴ The variation in the reported prevalence in these studies most likely reflects differences in the populations screened. Part of these differences might also be explained by the methods used to detect microscopic haematuria (dipstick versus microscopic analysis of the urinary sediment).¹¹ Mohr and co-workers found a prevalence of 13% in a population that consisted of men older than 35 years of age and postmenopausal women.¹⁵ Britton reported the presence of occult haematuria in 20.1% of men over 60 years of age undergoing screening for bladder cancer using dipstick tests.¹⁶ Various studies have shown that the prevalence of asymptomatic haematuria increases with age,^{16,17} whereas others found no correlation between advanced age and the prevalence of occult haematuria.¹⁵

THE DIPSTICK TEST

Evaluation of a urinary specimen should start with a dipstick test. The dipstick provides semiquantitative information about the presence of different components in the urine, such as erythrocytes, leucocytes and protein. Most tests are able to detect the presence of 25 to 50 erythrocytes per μ l urine which is equivalent to 2 to 3 erythrocytes per high power field on microscopic examination.¹⁸ The test

has a sensitivity of 93 to 96% and a specificity of about 60 to 80% for detecting erythrocytes in the urine.¹⁸⁻²⁰ The negative predictive value of the test is about 98%.¹⁹ Because of its high sensitivity, the chances of finding erythrocytes in a urinary sediment are limited when the dipstick test is negative for erythrocytes. Therefore, urinary specimens need no further examination for haematuria when the dipstick test for erythrocytes is negative.^{18,19,21} The number of urinary samples that can be discarded for microscopic examination in this way also depends on the prevalence of haematuria in the test population. Due to its poor specificity, a considerable number of urinary samples remain containing little or no erythrocytes at all on microscopic examination despite a positive dipstick. Myoglobinuria, poorly washed glassware, the presence of oxidising agents in the urine (e.g. povidine) and bacterial contamination are common causes of a false-positive dipstick test, whereas urinary samples that contain ascorbic acid or formaldehyde, or have low pH (<5.1), can lead to a false-negative dipstick test.^{1,11,18}

URINARY SEDIMENT

Examination of the urinary sediment allows differentiation between glomerular and nonglomerular forms of haematuria based on three distinguishing features. Firstly, the most convincing evidence for the presence of glomerular haematuria is the finding of erythrocyte and/or haemoglobin casts in the urine.²² In the thick ascending limb of the loop of Henle a mucoprotein is secreted called uromodulin or Tamm Horsfall protein. In urine that is sufficiently concentrated (specific gravity >1.010 g/ml) and acidified (pH <6), this mucoprotein will change into a gelatinous substance that will take on the contour of the tubular lumen.²³ All cells and proteins present in the filtrate at that time will be caught in the forming cast. The formation of the casts will be completed in the common collecting duct. Cells that appear in the urine at a later stage (for instance in the ureter or the bladder) cannot become incorporated into the cast. Thus all cells within casts must have originated from the nephron, and are therefore proof of the glomerular leakage of cells.

Secondly, erythrocytes in patients with glomerular haematuria have an aberrant shape compared with erythrocytes in a peripheral blood smear.^{4,5,22} These so-called dysmorphic erythrocytes appear in the urine when the physiological barrier of the glomerulus for the passage of cells is disrupted. This barrier is composed of capillary endothelium, glomerular basement membrane and an epithelial layer (the podocytes). When it has lost its impermeability for erythrocytes, these cells follow the urinary flow along the tubular system. During their course, the erythrocytes undergo alterations in shape. An *in vitro* study showed

that osmotic changes alone did not lead to the formation of dysmorphic erythrocytes, whereas a change in urinary osmolality in combination with a haemolytic environment did.²⁴ After this change of shape has taken place, erythrocytes cannot return to their original shape and thus appear as dysmorphic erythrocytes in the urine.

Acanthocytes or GI cells are erythrocytes with blebs and bulbs on their membrane. Some authors consider the presence of acanthocytes more specific for glomerular disease than dysmorphic erythrocytes.^{25,26} In a study by Dinda *et al.*, the presence of >4% acanthocytes in the urine resulted in both a specificity and sensitivity of 100% for diagnosing glomerular disease compared with 100 and 90%, respectively, for dysmorphic erythrocytes.²⁵ However, they observed a mean percentage of acanthocytes in their patients with glomerular disease of 20.6% of all red cells, a figure that is exceptionally high. In our experience, finding more than 4% of acanthocytes in a urinary sample is uncommon, even in patients with glomerular disease. We therefore regard acanthocytes as a subtype of dysmorphic erythrocytes and count them as such. Finally, the urinary sediment of patients with glomerular haematuria is characterised by the presence of a large variety of erythrocyte shapes. There is heterogeneity in shape and size of the erythrocytes, and also cell fragments can be found. Usually, at least three different shapes of erythrocytes are present in the urine of patients with glomerular disease, giving rise to a polymorphic picture (*figure 1*).²² This is in contrast with nonglomerular haematuria in which all red cells are similar in shape (isomorphic), resulting in a monomorphic pattern (*figure 2*).²²

Although there is no consensus in the literature on the upper normal limit of erythrocytes in a urinary specimen, most investigators consider two to three red cells per high power field to be the upper limit of normal.^{11,18,22,23,27} We consider urinary samples that contain three or more erythrocytes per high power field to be pathological, and

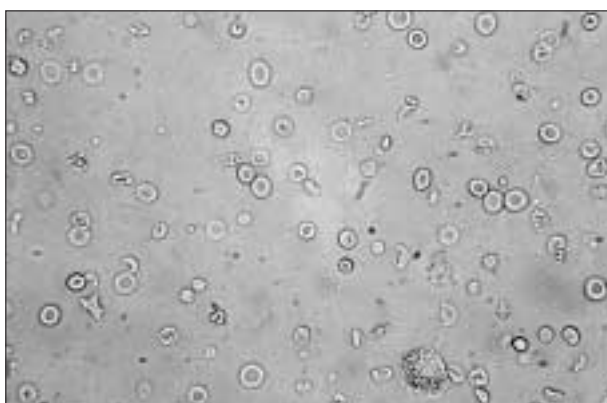


Figure 1
Glomerular haematuria - dysmorphic erythrocytes in a polymorphic pattern

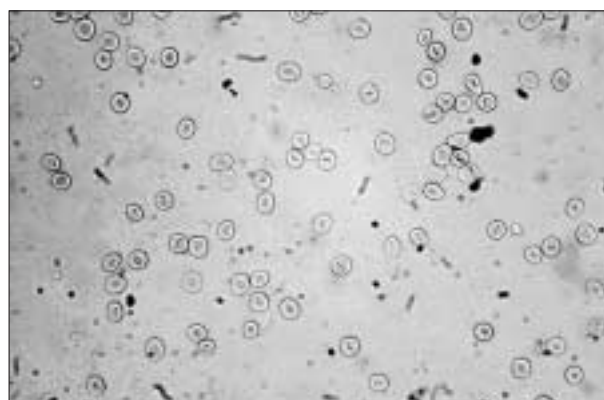


Figure 2
Nonglomerular haematuria - isomorphic erythrocytes in a monomorphic pattern

examine them for erythrocyte morphology. We then estimate the percentage of dysmorphic erythrocytes, after evaluating 100 red cells in the urinary sample. The study by Birch and Fairley did not delineate the percentage of dysmorphic urinary red cells required for the sample to be classified as dysmorphic and subsequent reports have varied on this issue with figures from 20 to 80%.^{18,22,25} However, in a previous study of fresh urinary sediments from 107 patients with known urological or nephrological causes of haematuria, we found that a value of 40% dysmorphic erythrocytes was a reliable cut-off point for differentiating between glomerular and nonglomerular haematuria.²² At this value, the sensitivity for diagnosing a urological cause of the haematuria was 100% and the specificity 66.7%. By including the absence of erythrocyte or haemoglobin casts as an additional parameter, the specificity rose to 88.1%. Consequently, we consider samples with more than 40% dysmorphic erythrocytes to be suggestive for glomerular haematuria, and definitely glomerular when the dysmorphic pattern is accompanied by the finding of erythrocyte or haemoglobin casts. When monomorphic haematuria is accompanied by erythrocyte or haemoglobin casts, a combination of a urological and a nephrological cause of the haematuria should be suspected. To summarise, glomerular haematuria is characterised by the presence of erythrocyte casts with more than 40% dysmorphic erythrocytes in a polymorphic pattern, whereas in nonglomerular haematuria, less than 40% dysmorphic red cells are found, a monomorphic pattern exists and erythrocyte casts are absent.

Phase-contrast microscopy is believed to be a superior method to bright-field microscopy for detecting dysmorphic erythrocytes.³⁵ Phase-contrast microscopy showed a sensitivity of 90% and a specificity of 100% for detecting glomerular haematuria, compared with 82 and 100%

with bright-field microscopy, when a cut-off point of 20% dysmorphic erythrocytes was used as indicator for glomerular haematuria and renal biopsy was used as the gold standard for diagnosing glomerular disease.²⁵ Provided that the settings of a light microscope are optimal (with lowering of the condenser lens), bright-field microscopy and phase-contrast microscopy appeared equally effective in differentiating glomerular from non-glomerular haematuria.²⁸ We prefer the standard light microscope because it is easily accessible and can be used in every reasonably equipped laboratory. Examination of a urinary sediment by standard light microscopy is also easier to perform and less time-consuming compared with phase-contrast microscopy. Inter-observer variability in the examination of urinary sediments is considered a major limitation for both forms of microscopy. In a study by Raman *et al.*, two independent observers differed in their interpretation of dysmorphic erythrocytes on 38% of occasions using phase-contrast microscopy.²⁹ However, the second observer had only limited experience, and unfortunately the correlation did not improve during the course of the study. After sufficient training of both observers, we found an excellent inter-observer variation using bright-field microscopy (correlation coefficient 0.90, kappa: 0.77).²² In a prospective study, trained laboratory personnel came to a different observation in 26% of 115 samples compared with an experienced nephrologist. This resulted in a difference in conclusion on the source of the haematuria in only 4% of cases (unpublished data). We therefore believe that after special training, an excellent agreement in the examination of the erythrocyte morphology can be reached between different observers.

FIXATION OF THE URINARY SEDIMENT AND CENTRAL EXAMINATION

As stated before, ample experience is necessary for adequate evaluation of the urinary sediment. Expertise is difficult to obtain because most physicians see too few patients with unexplained haematuria, and they therefore only seldom examine urinary specimens for erythrocyte morphology. Furthermore, we have observed that without special training the routine examination of urinary sediments by laboratory personnel is not reliable for differentiating between glomerular and nonglomerular haematuria [unpublished data]. As a consequence, microscopic evaluation of the urinary sediment is often lacking in the diagnostic work-up of unexplained haematuria. For several years now, a method of fixation of the urinary specimen is easily available.³⁰ A formaldehyde-containing fixative solution called CellFIX™, also used for the fixation of mononuclear cells in flow cytometry, proved useful for the fixation of urinary elements. We assessed this method

of preservation in 46 patients referred for the analysis of asymptomatic haematuria to a urology department.³⁰ Part of a urinary sample was studied within three hours after voiding, and another part was added to a small container filled with 0.2 ml of CellFIX™. The fixed sample was kept for ten days at room temperature before examination. Both samples were evaluated for red cell morphology and scored for the presence of casts. There was a highly significant correlation between the percentage of dysmorphic red cells in the fixed and fresh samples ($r=0.87$, $p<0.0001$). The mean difference of the percent scores of dysmorphic erythrocytes was $2.9 \pm 10.5\%$, which was not significantly different from zero (figure 3). Furthermore, no difference in the presence of casts was observed between the fixed and the fresh samples. Taken together, this fixation technique makes it possible to keep the specimen at room temperature for a minimum of ten days without changes in numbers or morphology of red cells and casts.

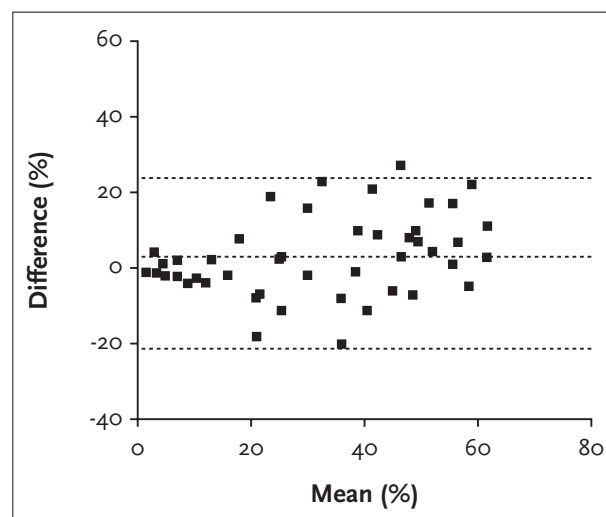


Figure 3
Percentages of dysmorphic erythrocytes in fresh and fixed sediments (n=46)

Mean scores of fresh and fixed against their difference (broken lines are mean \pm 2 SD).

The preparation of fixed urinary sediment is a simple procedure that can easily be performed at the general practitioner's office or in laboratory facilities that offer diagnostic services. Subsequently, these samples can be sent to an experienced laboratory, or be stored until examination by experienced individuals is available. Detailed instructions for making a fixed urinary sediment can be found in table 1. The examination of urinary samples in central laboratories with sufficient expertise will lead to a more effective diagnostic route for patients with asymptomatic haematuria.

Table 1
Directions for fixation of urinary sediment

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|---|
| Use a fresh urinary sample, preferably from a midstream collection. Storage of the sample for more than two hours at room temperature makes the interpretation of the urinary sediment difficult or impossible. |
| Centrifuge 10 ml of urine in the usual way: 1500 rpm (300 g) for three minutes will suffice. |
| Decant the supernatant and transfer four drops of the sediment (about 0.2 ml) to a small plastic vial that has been pre-filled with 0.2 ml Cellfix™ solution (in a 1:10 dilution with demineralised water). |
| Screw the cap tightly. |
| Thereafter the sample can be stored for at least ten days at room temperature and be sent to a specialised examiner by regular mail. |
| Do not store the fixed sample in a refrigerator or icebox. |

Cellfix™ is a formaldehyde-containing fixative that was used for the fixation of mononuclear cells in flow cytometry (it is produced by Becton Dickinson).

RECOMMENDATION

Based on the fact that the prevailing diagnostic algorithms for the analysis of asymptomatic haematuria are not very efficient and that a considerable number of patients undergo inappropriate urological evaluation, we make a plea for a diagnostic strategy that includes the examination of the urinary sediment. Since the urinary sediment has proven to be a suitable diagnostic tool in differentiating glomerular from nonglomerular haematuria, it should be used early in the diagnostic route of patients with

asymptomatic haematuria. We therefore recommend that patients who present with asymptomatic haematuria follow the algorithm displayed in *figure 4*.

CONCLUSION

Examination of the urinary sediment is a simple, cheap, noninvasive and reproducible test with a great discriminatory potential to differentiate between glomerular and nonglomerular forms of haematuria. Someone with suffi-

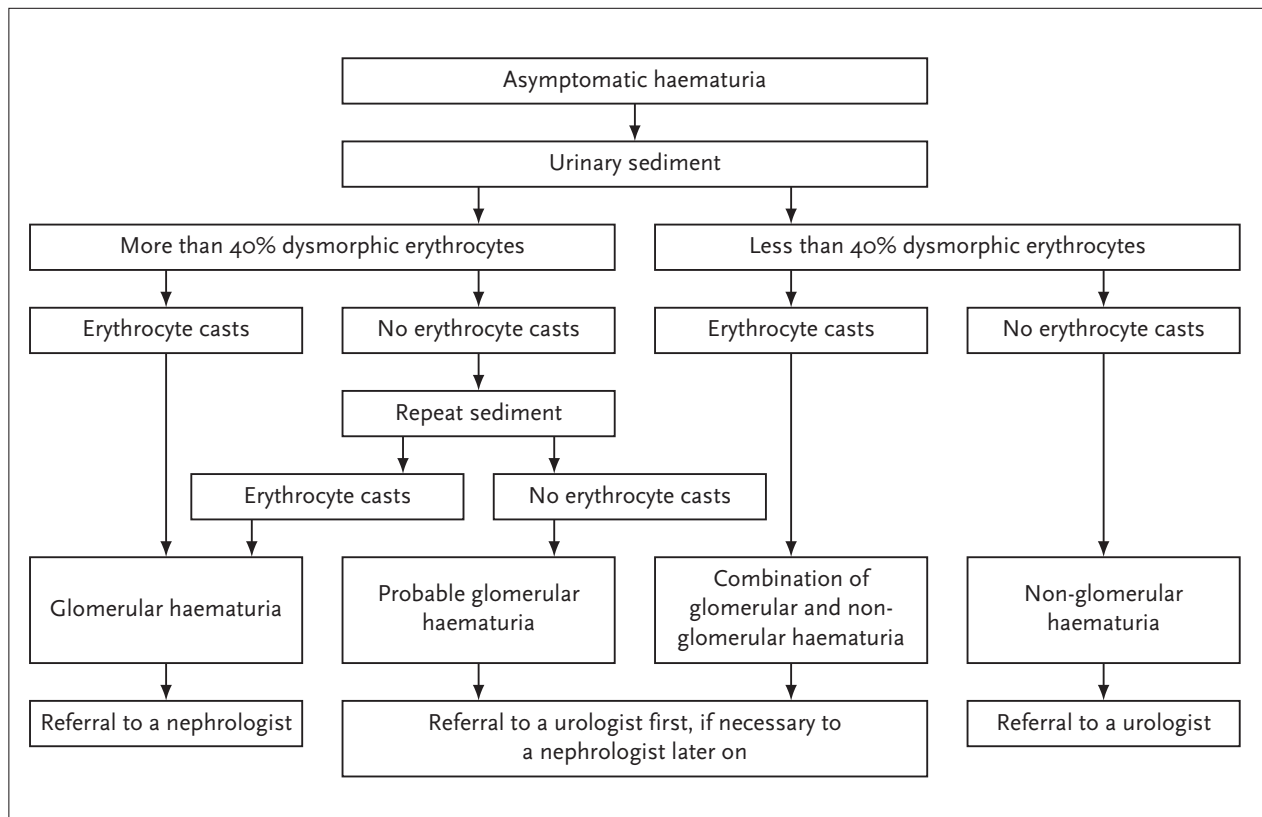


Figure 4
Diagnostic algorithm for the analysis of patients with asymptomatic haematuria

cient expertise, for example a specially trained technician in a central diagnostic laboratory, should perform it. Ideally, examination of the urinary sediment should be done before referral of the patient to a medical specialist.

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