SEED Urinalysis

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Laboratory investigation of haematuria

Apart from diagnosing possible urinary tract infections, haematuria is one of the most frequent findings on urinalysis. The presence of erythrocytes in urine can be entirely physiological. According to literature, the number of excreted erythrocytes can amount up to 3,000 or 20,000 erythrocytes/mL of normal urine or up to 3 x 10^6 erythrocytes/24 h collected urine. Approx. 10% of healthy people have even higher levels, thus markedly exceeding the defined normal laboratory ranges. The normal ranges given in the literature for erythrocytes in sediment microscopy also differ and the reported figures range between 2–3 erythrocytes/high power field and up to 10 erythrocytes/high power field, which can be explained by the different methods of obtaining and counting samples.

If a haematuria is pathological, there are many possible reasons for this. These may include coagulation disorders and renal or urinary tract disease. Thus, it is essential to investigate every case of haematuria.

The initial identification of haematuria – macroscopic or by means of test strips

Haematuria of either physiological or pathological origin can vary in degree: macrohaematuria (over approx. 0.5–1 mL blood/litre urine results in macroscopically obvious reddening of the urine) is usually noticed by the patient and reported to the clinician, whereas the test strips used routinely for the first laboratory screening test with their relatively high sensitivity are often the first evidence of the presence of undetected and asymptomatic microhaematuria (up to 0.5–1 mL blood/L urine). However, one test strip result on its own does not provide any information on whether the positive result indicates the presence of haemoglobin, myoglobin or erythrocytes. Subsequent microscopy is therefore required.

If no erythrocytes are found on microscopy, haemoglobinuria and myoglobinuria can be confirmed by further laboratory tests

- Haemoglobinuria is present when increased haemolysis takes place in the blood. Some of the free haemoglobin has been excreted in the urine and was detected by the test strip. In the blood, on the other hand, the increased tendency to haemolysis can be underpinned diagnostically by the finding of a raised serum LDH or reduced serum haptoglobin level.
- Myoglobinuria occurs together with myoglobinaemia when muscle tissue is destroyed. In the serum there are then higher levels of creatine kinase, which is released from muscle cells when muscle is damaged.

If no erythrocytes are found on microscopy, the possibility of lysis of the erythrocytes in the urine itself should be considered besides the conditions described above. Increased lysis of erythrocytes occurs in urine with a specific gravity below 1.010 and alkaline pH. An alkaline milieu can cause swelling of the outer membrane layers of the erythrocytes, thus causing the formation of echinocytes [1]. First the cells swell because of the osmotic ratio and then the haemoglobin gradually leaks out of the erythrocytes. Lysis processes are observed if the sample remains at room temperature for some time since the pH can become alkaline due to the growth of bacteria and the associated metabolic activity, or if the urine had already been in the bladder for a prolonged period prior to micturition.

Peroxidase-positive bacteria can also lead to a colour change in the urine test strip pad for blood even when there are no erythrocytes in the urine. This means a false-positive result for blood in the urine.

Microscopic detection of haematuria

If erythrocytes are found on microscopy, a positive result should be confirmed in at least two to three urine sediments at intervals of a few days, as haematuria can also be intermittent. The question then is whether

- pathological haematuria is present and
- whether localisation of the site of bleeding is possible.

Temporary haematuria can be observed, for instance due to mingling of menstrual blood, as a concomitant finding with urinary tract infections, or after physical exertion by healthy persons. In such exertional haematuria, the erythrocytes in the urine may derive from alterations in the permeability of the glomeruli, or from renal microtraumata; however, they can also have a non-glomerular origin (e. g. microtraumata to the mucosa of the apex vesicae, prostate gland or urethra). If the exertional haematuria disappears spontaneously within 24 to 72 hours, no further investigations are necessary apart from the exceptions listed below.

If macrohaematuria, persistent haematuria or recurrent haematuria is observed following haematuria caused by exercise, this may indicate a urological disorder. If exertional haematuria occurs in men aged over 45 years, there should always be further investigation of the possible causes [2].

If erythrocytes are present in urine without detectable proteinuria or erythrocyte casts, this is called ‘isolated microhaematuria’. It is found in 1–4 % of the and about 2.6 % of pregnant women. Non-invasive laboratory methods are used primarily for further investigation of possible bleeding sources, such as microscopic assessment of erythrocyte morphology and analysis of urinary proteins.

Microscopic detection of haematuria

If possible, erythrocyte morphology should be assessed using phase contrast microscopy, which allows the best evaluation of abnormal morphology. After glomerular passage, the erythrocytes exhibit typical morphological changes. Besides ring forms with a smooth and wavy structure and deformed or dysmorphic glomerular erythrocytes, sometimes with inner and outer spicules, acanthocytes (known as ‘Mickey Mouse erythrocytes’) are a special form of dysmorphic erythrocytes with vesicle-shaped projections. Assessment of the erythrocytes requires practical experience and a good knowledge of urine microscopy:

1. Glomerular erythrocytes are typically smaller than erythrocytes of non-glomerular bleeding sources.
2. The erythrocytes from glomerular sources of bleeding vary in shape and size, whereas erythrocytes in haematria of non-glomerular origin are uniform in size and shape.
3. Erythrocytes of glomerular origin have often lost haemoglobin.
4. In glomerular haemorrhage, erythrophagocytes can sometimes be seen microsc opically also. Erythrophagocytes are tubular epithelial cells that have already absorbed erythrocytes [3], [4].

In postrenal haemorrhage, in contrast, the erythrocytes largely preserve their shape (designated as eumorphic, isomorphic or non-glomerular erythrocytes).

Sysmex customers can use the knowledge of the described typical size characteristics and size variability in haematuria when they use a urine fluorescence flow cytometer (Sysmex UF-series) in their laboratory. If haematuria is detected, the size and size distribution of the erythrocytes are analysed automatically. The additional RBC information in the UF-series states whether the erythrocytes are normal and uniform in size (the RBC info then states: ‘isomorphic?’) or whether any dysmorphic erythrocytes are present (the RBC information then states either ‘dysmorphic?’ in the presence of small erythrocytes or ‘mixed?’ if erythrocytes with a noticeable variation of size are present). Microscopy and confirmation of dysmorphic erythrocytes can then be limited to samples in which the morphology was assessed as abnormal. The quality of the RBC info on the UF machines has demonstrated relatively high reliability in sorting out those cases of haematuria in which no dysmorphic erythrocytes are found.

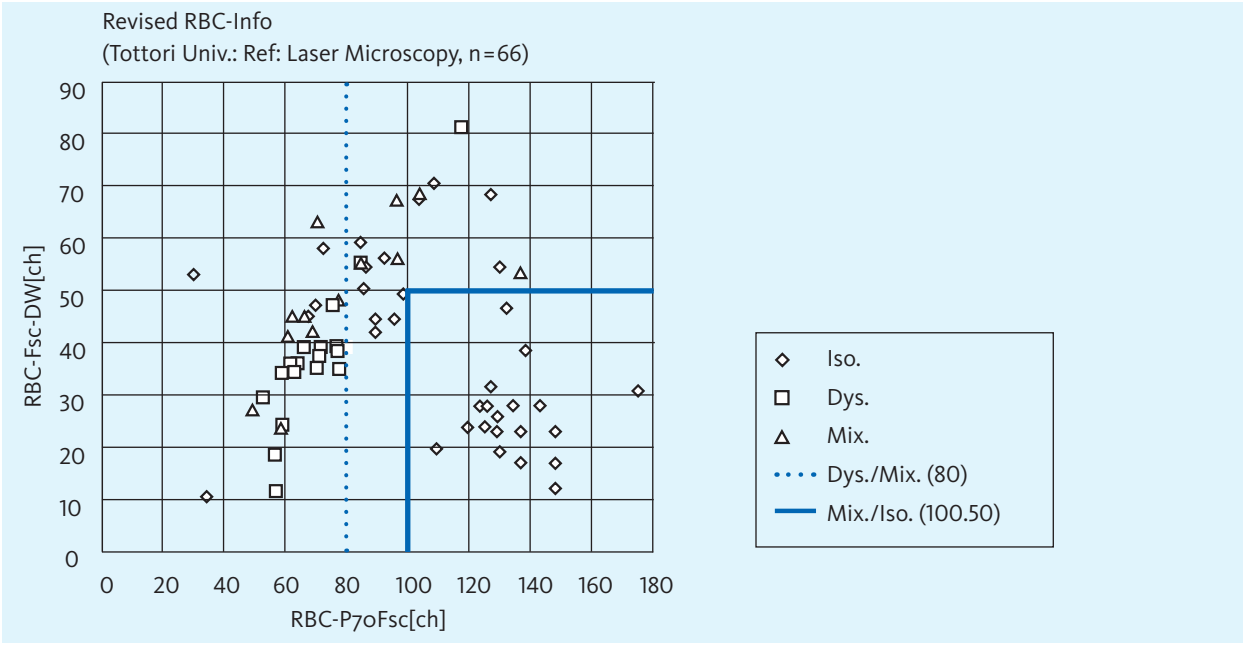

Fig. 1 *The RBC info 'isomorphic?' issued on the Sysmex UF-series, identified by the blue marking in the right lower quadrant, compared with assessment of the erythrocytes using laser microscopy (see top symbol in the legend on the right of the figure).*

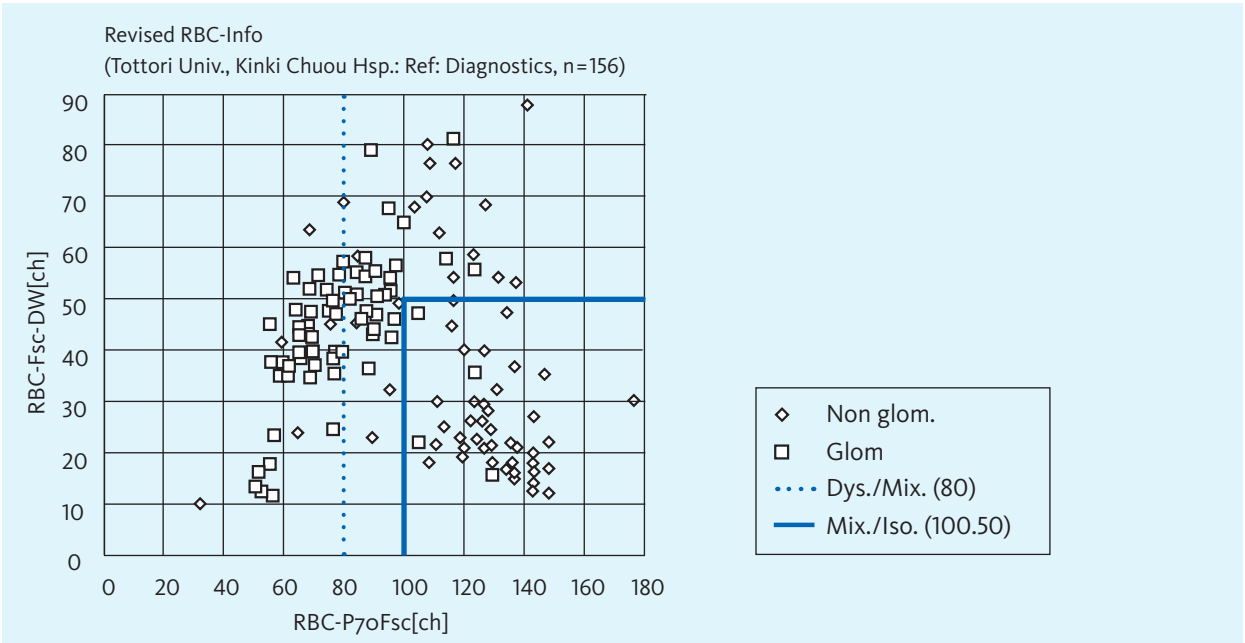

Fig. 2 *The RBC info issued on the Sysmex UF-series compared with localisation of the bleeding source (see the top two symbols in the legend on the right of the figure)*

Detection of predominantly dysmorphic erythrocytes suggests a renal cause of the haematuria (glomerular haematuria). If no dysmorphic erythrocytes are present, however, glomerular haemorrhage cannot be ruled out.

The family history and clinical findings play an important part along with the laboratory steps listed above, particularly in children, since it is desirable to avoid invasive investigation methods as far as possible [5]. If dysmorphic haematuria is found repeatedly, both in isolation and in combination with proteinuria and casts in the urine, the familial frequency of haematuria is then enquired about through the family history, and likewise systemic diseases with renal involvement can also be ruled out; this may involve serological tests as well as further physical examinations. Typical other routine diagnostic tests include measurement of creatinine, urea,

creatinine clearance and total protein, measurement of albumin using wet chemistry and further tests such as the C3 complement test to investigate a suspected diagnosis of immune complex diseases such as systemic lupus erythematosus, and the ASL titre (antistreptolysin titre) to detect a postinfectious status or existing infection due to streptococci, especially beta-haemolytic streptococci. Particularly in children, the antibodies produced after infections by streptococci with their nonspecific antigens can attack the heart valves and brain structures and also glomeruli [6].

Further tests for investigating haematuria are lupus serology, hepatitis serology and molecular genetic tests or HIV tests [6].

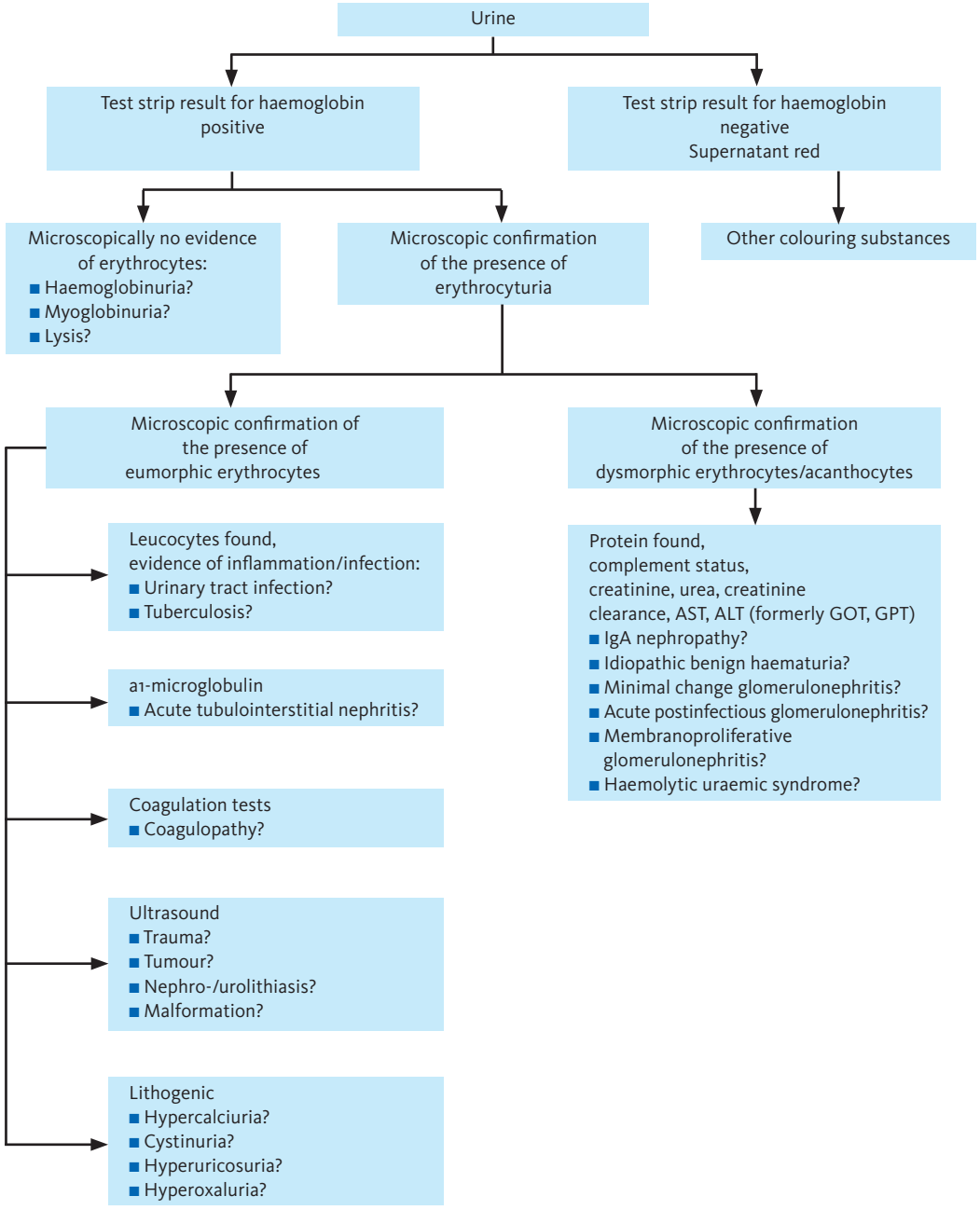

Fig. 3 *Procedure with haematuria*

In children, Alport's syndrome or thin basement membrane syndrome are found in nearly 80 % of recurrent or persistent haematurias without dysmorphic erythrocytes [5]. In general, the most common cause of isolated dysmorphic microhaematuria in approx. 2/3 of the cases is nonspecific chronic alterations of the renal corpuscles (usually IgA nephropathy). In approx. 1/4 of the cases there is nephropathy of the thin basement membrane type, and no cause can be found in the remaining cases [7].

If haematuria is found without evidence of dysmorphic erythrocytes and thus no evidence in the urine of renal involvement (though this can nevertheless be present in rare cases), culture is started if there are other signs of inflammation such as the presence of leucocytes and bacteria or mycosis. The haematuria can be due to inflammation-induced changes in the mucosal perfusion in the urinary tract. Vasodilatation can allow erythrocytes to pass from the vessel lumen into the urine. Most cases of haematuria without evidence of glomerular erythrocytes are caused by inflammation in the urogenital tract.

If there are no signs of a urinary tract infection, other possibilities should be considered, such as the presence of a urinary calculus, which can cause erythrocytes to pass into the urine as a result of mechanical irritation or blood vessel injury.

Besides imaging, calcium and creatinine are often measured. Urolithiasis can also occur in children, which justifies appropriate investigations. While the prevalence of hypercalciuria is very low in healthy children at 2.9 to 6.2 %, a raised calcium/creatinine index is found in 30 % of cases of children with isolated haematuria after other urological and nephrological causes have been ruled out. More than 10 % of the children develop urolithiasis within 4 years [8].

In patients over 45 years, especially male patients with an increased risk of disease, tumour as the cause of haematuria without glomerular erythrocytes must be ruled out. Tumours, especially malignant ones, have their own blood supply, which often has defects in the vessel walls because of the relatively simple structure. This results in bleeding. However, the haematuria can also be due to growth of a tumour into the mucosa of the urogenital tract.

Even though there are many reasons for haematuria, approx. 10 % of all cases remain unexplained and are diagnosed as idiopathic haematuria.

Ultimately, most cases of haematuria prove to have a harmless cause. However, the possibility of renal involvement and neoplasia should always be considered in certain age groups or with certain symptoms. Laboratory investigation is the first step toward specific therapy.

References

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