Laboratory Case Report

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Double false-negative traps in urine routine test: a case report

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Abstract

Background: Urinalysis is one of the most commonly performed tests in clinical practice and supplies important information for a series of clinical conditions, including renal and urinary tract diseases. The clinical laboratory often completes urinalysis through the combined use of urinary dry-chemistry and formed-element analyzers. Urine red blood cell (RBC) morphology test is often used to discriminate the source of hematuria by manual microscopy.

Case presentation: In this case report, we describe a 39-year-old woman with chronic glomerulonephritis (CGN) who underwent both urine routine test and RBC morphology test. Her RBC count was in the normal range and the occult blood test was negative in routine test, while the RBC morphology test indicated the presence of non-homogeneous hematuria.

Conclusions: Therefore, we analyzed the causes of false-negative result on the urine chemical analyzer and the automatic microscope system, respectively.

Keywords: automatic microscope system; chronic glomerulonephritis; false negative; occult blood test; RBC morphology; urinalysis.

Introduction

Chemical and microscopic testing of urine samples, known as urinalysis, is one of the most important routine tests in clinic. It is mainly used for the diagnosis, differential diagnosis, treatment monitoring and health

screening of urogenital, metabolic, hepatobiliary and other systemic diseases. Microscopic testing is the gold standard for red blood cell (RBC) morphological test. The morphology of RBCs in urine is an important reference value in distinguishing glomerular hematuria from non-glomerular hematuria. In clinical practice, the combined use of urine routine test and urine RBC morphology test can help improve the RBC detection rate and avoid misdiagnosis and missed diagnosis.

Chronic glomerulonephritis (CGN) is a group of glomerular diseases with proteinuria, hematuria, hypertension and edema as the basic clinical manifestations. The onset way of CGN is different and the disease progress is slow. CGN can have varying degrees of renal function decline. With a tendency to deterioration of renal function, it will eventually develop into chronic renal failure [1]. The main clinical manifestations may be different due to the pathological type and stage of the disease. Therefore, urine routine tests should be conducted regularly to monitor the progress of the disease by observing the count and morphology of RBC.

Case presentation

A 39-year-old female with CGN was followed up regularly at the nephropathy clinic of the First Affiliated Hospital of Nanjing Medical University for urinalysis. The fresh mid-stream urine sample was first collected in a disposable container and then randomly poured into two test tubes for routine and manual microscopic examination, respectively. One urine sample was placed in a rack and introduced on the automated urine test-strip analyzer (iChem Velocity: Beckman, Chatsworth, CA, USA) for chemical test, then it was transported to the automated intelligent microscopy analyzer (iQ200: Beckman, Chatsworth, CA, USA) automatically for visible component analysis. The other sample (10 mL) assessed by manual microscopy was centrifuged at 400 g (=1350 rpm) for 5 min, supernatant urine was removed, and the sediment was carefully re-suspended with a pipette in the remaining 1 mL of urine. Aliquots of the suspension (1 µL) were filled into a urine sediment

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counter for manual microscopy and then the information about the number and morphology of RBCs was recorded. The chemical results of urine showed that hemoglobin was negative, urine protein $\pm$, ascorbic acid 2+ and there was no special changes in other indicators. Classification and counting results of automatic microscopy showed the following: RBC 13/ μ L, white blood cells (WBCs) 15/ μ L (reference interval: RBC 0–17/ μ L, WBC 0–28/ μ L), few squamous epithelial cells, no cast or bacteria. However, the presence of RBCs in urine sediment was clearly observed by manual microscopy (NIKON 50I phase-contrast microscope: Nikon, Tokyo, Japan, NIKON ECLIPSE E100 bright field microscope: Nikon, Tokyo, Japan). The RBC count was 60/ μ L, and 70% of them were smaller in diameter than normal RBC. As the two samples were from the same urine source, we suspected that the results of the automated urine system might be false-negative in the RBC examination.

Next, we performed a series of verification and analysis work. The urine sample that was originally examined on the automated urine system was retested by an occult blood duplex dipstick (NewScen Coast, Tianjin, China) and its sediment was observed by manual microscopy.

Figure 1: Immunological method.

The test line (T) and the control line (C) show a color band at the same time, and the result is positive. Chemical method: the color of the paper changing from orange to yellowish green or dark green within 1 min after adding the sample is positive. No discoloration within 1 min is negative. In this case, it showed that the immunological method was positive, while the chemical method was negative.

The duplex dipstick combines immunoassay with the chemical method and can detect human hemoglobin in urine samples. At this time, the colloidal immunization method was positive for human hemoglobin, while the chemical method was still negative (Figure 1). Therefore, we inferred that the chemical result of occult blood was false negative due to the influence of ascorbic acid. In the meanwhile, a large number of small RBCs with a diameter of about 6.3 μ m were found in the unclassified list of IQ200, and the presence of RBCs in urine was confirmed by manual optical and phase contrast microscopy, which indicated that the initial results of iChem VELOCITY and IQ200 were both false negative.

Discussion

Clinical laboratory often completes urinalysis through the combined use of urinary dry-chemistry and formed-element analyzers. The IQ200/iChem workstation mentioned in this case is one of the representatives.

The urinary dry-chemistry analyzer is mainly based on the measurement of light reflection. The underlying mechanism of occult blood measurement is that the ferroheme in the hemoglobin of RBCs has peroxidase activity and can catalyze the chromogenic reaction of a hydrogen donor [2]. Thus, some reducing substances like high concentrations of ascorbic acid in urine may have inhibitory effects and lead to false-negative results. Although the manufacturer claims states that ascorbic acid concentrations above 10 mg/dL can cause interference with hemoglobin, the extent and scope of the impact were not specified. Hence, we did an experiment on the effect of ascorbic acid on the hemoglobin assay. iChem Velocity dipsticks (Iris Diagnostics, Chatsworth, CA, USA) were used for chemical test of urine. Specific hemoglobin concentration of urine samples were prepared by adding appropriate volume of whole blood to the negative urine pool sample. Prepared urine samples with hemoglobin concentrations of 0, 0.015, 0.03, 0.05, 0.10, 1, 10, 100 and 1000 mg/dL were then spiked with ascorbic acid (20 and 40 mg/dL). Each urine sample with specific combination of hemoglobin/ascorbic acid concentration was tested in duplicate. The

Table 1: Effect of ascorbic acid on the determination of hemoglobin.

Hemoglobin concentration, mg/dL	0	0.015	0.03	0.05	0.1	0.5	1	10	100	1000
Ascorbic acid concentration, mg/dL/category	0/–	–	–	±	±	1+	2+	3+	3+	2+
	20/1+	–	–	–	–	±	1+	1+	3+	2+
	40/2+	–	–	–	–	±	1+	1+	2+	2+

user manual of iChem Velocity indicates that the measurement range of hemoglobin is 0.03– ≥ 1.0 mg/dL, and results of our study confirmed this claim (Table 1). If hemoglobin concentration exceeds the maximum detection limit of the strip, the result may maintain at the highest preset level (3+) or be random and unreliable. Ascorbic acid begins to interfere with hemoglobin at low concentrations (0.03 mg/dL), so it can easily cause false-negative results in occult blood test. Adriana Unic et al. [3] showed similar results in their study. The immunological method based on antigen-antibody reaction is more specific and free from the interference of ascorbic acid. When ascorbic acid is positive in urine, the immunological method can be used to verify the accuracy of the occult blood test.

Due to the increased number of daily samples and the need to shorten the turnaround time (TAT), a strategy combining primary screening using automated routine urinalysis technology with microscopic review has become a practical and feasible method. IQ200, a fully automated microscopic module of Iris, is based on digital imaging principles [4]. In this system, the formed elements in the urine pass by in a laminar flow through the objective lens of a charged coupling device video camera. Hundreds of captures of a digital camera are evaluated by intelligence identification software, and each particle is classified on the basis of some characteristics such as shape, contrast and texture of the particle. Any identified particles that do not fulfill predefined characteristics, according to predetermined rules for automated classifications, are reserved

in a list of unclassified cell images to be manually classified by experienced laboratory staff [5]. Previous studies have suggested that the analyzer showed similar performances and good compatibility to manual microscopy [6]; however, they are still inadequate in the determination of RBC in some pathological samples. In this case, the patient with CGN has a large number of small RBCs in urine (more than 70%), which are reserved in the unclassified menu. When the urine occult blood test was negative and the RBC count was in normal range at the same time, it is possible to ignore the careful identification of unclassified cells images, especially for inexperienced staff. Confirmation of pathological results of the automated system by manual examination may be needed [7].

It is known that hematuria can be classified as glomerular (the presence of dysmorphic red blood cells [dRBCs]) and non-glomerular (the presence of isomorphic red blood cells [iRBCs]) depending on the source of the bleeding [8]. dRBCs are first-line biomarkers for detecting glomerulonephritis (GN) in patients with hematuria [9]. Currently, phase-contrast microscopy (PCM) is the gold standard to identify dRBCs when distinguishing GN and is currently widely used. The better contrast between the background and the particles supplied by PCM improves visualization of the cells and their morphologic details (Figure 2). This is the advantage of PCM over bright field microscopy.

In conclusion, the use of automatic urinalysis conforms to the development of medicine, meets the demand

Figure 2: Small red blood cells.

(A) Bright field, (B) phase-contrast microscopy (all images: original magnification, $\times 400$). (C) A large number of small red blood cells with a diameter of about $6.3\ \mu\text{m}$ were found in the unclassified list of IQ200.

of clinical mass specimen detection and liberates the labor force. However, interference factors may lead to false results due to methodological limitations, and automated urinary microscopy is still inadequate in identifying pathologic cells. Standardized manual microscopic detection is still the reference method for urinary sediment analysis and the key to break the double false-negative trap.

Patient follow-up

We contacted the patient after this urine analysis and got some clinical medication information with her consent. She took cordyceps militaris tablets regularly according to the doctor's advice to treat chronic renal insufficiency and panax pseudo-ginseng powder (a kind of Chinese herbal medicine) on her own for stopping renal bleeding. She also took vitamin C intermittently to boost immunity. We recommended the patient stop taking vitamin C and eat less fruits and vegetables that are rich in vitamin C before the next urine test. Forty-seven days later, her urine analysis result showed that ascorbic acid was negative, and occult blood test 3+. The initial RBC results of IQ200 were 50/ μ L, and there were still a large number of small RBCs in the unclassified list. The final RBC count was 113/ μ L confirmed by manual microscopy.

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Ethical approval: The local Institutional Review Board deemed the study exempt from review.

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