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More than meets the I(ris): Use of manual urine microscopy to complement automated findings in acute kidney injury

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ABSTRACT

Evaluation of patients with acute kidney injury requires comprehensive assessment that includes a urinalysis, which features both semi-quantitative assessment with a urine dipstick and urine microscopy. This process is labor intensive for clinical laboratories, and availability of excellent automated instruments for urinalysis has prompted utilization and acceptance of this strategy by both by laboratories and clinicians. Recently, however, interest in provider performed microscopy has enjoyed a renaissance thanks to both improved microscopy techniques and the endorsement from social media in nephrology. Here, we present two cases of acute kidney injury in which manual microscopy added valuable information to the automated microscopy.

Diagnosis and care of patients with acute kidney injury requires synthesis of the clinical history, physical examination and an array of laboratory data that includes chemical analysis of the blood and urine along with urine microscopy [1]. Recently, many in the nephrology community have shared renewed interest in urine microscopy using an array of techniques that include phase microscopy and point-of-care urine sediment staining [2]. This strategy is labor intensive but can complement the work done in the clinical laboratory. Here, we present two complex cases of acute kidney injury in which information gleaned from manual microscopy of the urine added valuable information to the automated microscopy and traditional markers.

Case 1

A 57-year-old man with type 2 diabetes mellitus and stage 3 chronic kidney disease presented with fever and back pain of 3 weeks duration. *Enterococcus faecalis* grew from the blood and did not clear with appropriate antibiotic therapy. A transthoracic echo demonstrated native aortic valve endocarditis and severe aortic regurgitation. His initial creatinine was 3.1 mg/dl which improved with intravenous fluids but then worsened without a clear instigating event. Nephrology was consulted. The fractional excretion of sodium (FENa) was 0.6%. The urinalysis was notable for hematuria and proteinuria and the urine sediment showed many dysmorphic red blood cells (RBC's), suggestive of a glomerular source [3]. Complement levels were normal. A kidney ultrasound was normal. Supportive care was provided and on the 11th hospital day, he underwent aortic valve replacement complicated by intraoperative hypotension requiring vasopressor therapy. His creatinine rose further. Repeat FENa was now 1.5%. The repeat automated urinalysis showed persistent proteinuria, but no casts were identified. Manual urine microscopy now featured nondysmorphic RBC's and

Abbreviations: AIN, Acute interstitial nephritis; CLIA, Clinical Laboratory Improvement Amendment; FENa, Fractional Excretion of Sodium; FOAMed, Free Open Access Medical Education; RBC, Red Blood Cell; WBC, White Blood Cell.

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numerous “muddy brown casts” (ie. pigmented granular casts) (Fig. 1).

In this case, the differential diagnosis of acute kidney injury was broad both at presentation and then following surgery. Given the cardiac dysfunction, reduced kidney perfusion could have led to prerenal azotemia as suggested by the low FENa early in his clinical course. He also had glomerular hematuria which can occur with infection associated glomerulonephritis. The FENa is often low in this situation, though in addition, the serum complement levels are usually low. Endocarditis can cause embolic phenomenon, but this is usually either asymptomatic or leads to acute flank pain rather than kidney dysfunction. Ultimately, the repeat FENa was high and the urine sediment by manual inspection demonstrated many granular casts characteristic of acute tubular injury [4].

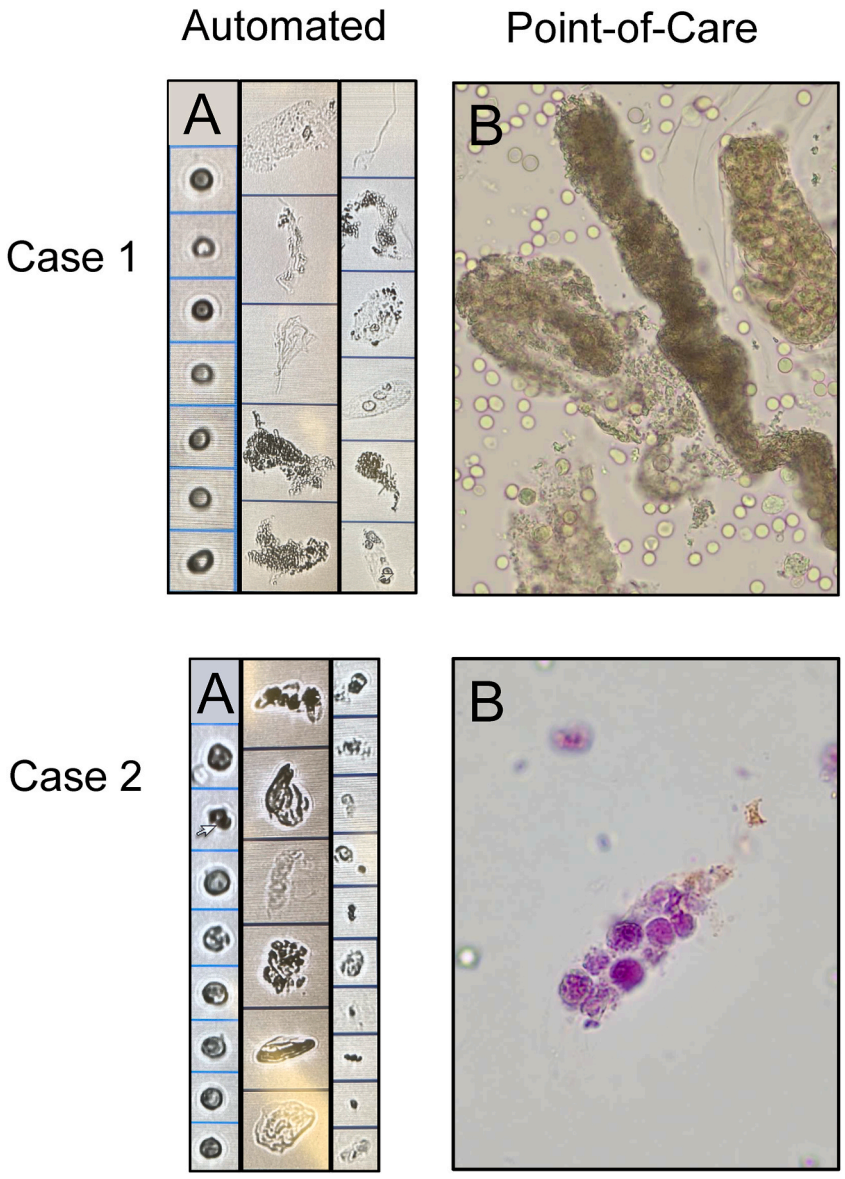

Fig. 1. Representative images of Urine sediment analysis using automated and manual point-of care techniques for cases 1 and 2. Urine specimens from each patient were divided into two containers and assessed simultaneously. One was analyzed using the Iris iQ200 Series Automated Urine Microscopy System analyzer (Iris Diagnostics, Beckman Coulter). Representative images from the “results screen” are show in Panels A. The second aliquot of each urine specimen was evaluated by point of care microscopy. 10 ml of urine was centrifuged at 400 × g for 5 min. Microscopic inspection of the urinary sediment was performed using an Olympus CX43 microscope, 40X objective, and photographed using an attached Olympus EP50 digital camera. Representative images are seen in Panels B. 1B is brightfield without stain. The sediment in 2B was stained for 1 min using Sternheimer-Malbin stain (Globe Scientific 3810, 1 drop added to resuspended sediment). In Panel 1A, numerous red blood cells (RBC’s) seen here in the left-hand column were identified in addition to many unclassified particles which would trigger manual review by a laboratory technician. 1B shows a pigmented granular cast and numerous nondysmorphic RBC’s. Panel 2A demonstrates white blood cells (WBC’s) in the left-hand column and unclassified particles. In 2B, by manual inspection, WBC casts were identified. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Case 2

A 67-year-old woman with a history of esophageal cancer was treated with immunotherapy for refractory disease. She also had a history of atrial fibrillation, hypertension, and a baseline creatinine of 1.2 mg/dl. An immune checkpoint inhibitor, pembrolizumab, was begun monthly 4 months prior and the last dose was administered two weeks earlier. She presented to the hospital with nausea, vomiting, and weight loss but no urinary symptoms. She was afebrile with normal blood pressure. Laboratory studies on admission were notable for a creatinine of 3.4 mg/dl, no leukocytosis or eosinophilia. She was initially treated with intravenous fluids with an improvement in her serum creatinine. FENa on admission was 0.6% and the automated urinalysis showed significant pyuria with two hyaline casts/low powered field whereas manual microscopy showed pyuria and white blood cell (WBC) casts (Fig. 1). Taken together, the clinical history of losses, low FENa and initial improvement in serum creatinine were consistent with a component of prerenal azotemia, but the WBC casts in the setting of a proton pump inhibitor and an immune checkpoint inhibitor raised concern for acute interstitial nephritis (AIN). Both the proton pump inhibitor and pembrolizumab were discontinued. She was treated with corticosteroids for presumed checkpoint inhibitor induced AIN and enjoyed improvement in her kidney function.

Evaluation of patients with acute kidney injury requires comprehensive assessment that includes a urinalysis, which features both semi-quantitative assessment with a urine dipstick and urine microscopy. This process is labor intensive for clinical laboratories and subject to a range of challenges that includes preanalytic factors related to collection, delivery to the laboratory, storage and preparation for analysis as well as analytic factors, particularly for the inspection of the urine sediment, which requires training and assessment of proficiency. Routine urine microscopy by laboratory personnel is challenging given the large number of samples, the time required to complete the test, and the fact that urine specimens are best evaluated within 2 h of specimen collection unless preservative is added [5]. Automated technology for urinalysis became available in 1985 and widespread use naturally followed. Use of automated techniques for urinalysis combined with manual inspection for elements that are not well characterized, has allowed laboratories to improve reproducibility and accuracy while decreasing turn-around-time [6].

Automated urinalysis is an excellent screening test for a range of kidney disorders and plays an important role in the assessment of AKI. The automated urinalysis interprets each colorimetric dye on the urine dipstick at the appropriate time interval and faithfully records the results. Next the automated instruments analyze the insoluble elements of the urine either by digital capture or flow cytometry. With the popular Iris iQ200™ Urine Microscopy System (Beckman Coulter Life Sciences), a high definition camara captures 500 images of the urine sample, the software categorizes the elements of the urine based on size, shape, texture and contrast, and the operator verifies the software categorization. With flow cytometers, urinary particles are labeled with fluorophores and then can be categorized by their fluorescence, size, impedance, and scattered light. Results are provided by scattergram rather than particle images [7]. Both modalities offer remarkable precision and accuracy in the total cell count of red and white blood cells but are less useful for assessing dysmorphic red blood cells, kidney tubular epithelial cells, casts, and certain crystals [8]. These elements are best evaluated directly with microscopy.

Despite this, it is worth noting that traditional methods of provider performed microscopy use 10X and 40X objectives with a 10X ocular lens and together, these yield images only slightly superior to those obtained from the automated instruments, though the provider may have the luxury of spending more time reviewing the urinary sediment than laboratory staff. Furthermore, the provider's impression of the urine may be colored by other findings; for example, a cellular cast in the setting of pyuria is more likely to be identified as a white blood cell cast (particularly if the cells inside are the same size those outside the cast matrix). Providers may also be influenced by the clinical history and the laboratory data. Even with these advantages, however, providers' assessment of urinary findings is subjective and interobserver agreement is less than ideal. Indeed, in one series, expert nephrologists from 15 US teaching hospitals demonstrated substantial differences in interobserver agreement; they only agreed on 59% of urinary casts [9].

Disillusionment regarding urine microscopy, the time required to process and review a single specimen and the useful information gleaned from automated urinalyses, make it easy to skip this time-honored tradition. Indeed, some nephrology training programs have eschewed the visual inspection of the urinary sediment as a core competency (S.H.L., personal communication). Nevertheless, provider performed microscopy can yield information that may contribute to understanding of a patient's diagnosis and potentially changing clinical course. Happily, recently, there has been a resurgence of interest in the urinalysis, and this flurry of attention stems from nephrology based social media interest and as part of the movement for excellent Free Open Access Medical Education (FOAMed) [10]. Increased interest has also been sparked by use of newer microscopes that offer a range of modalities for review, along with growing popularity of staining urine to improve identification of findings. For example, brightfield microscopy, when the specimen is illuminated from below, provides better resolution whereas darkfield, which excludes unscattered or direct light, can be helpful to view transparent structures like hyaline casts and particles with low resistive index such as lipids and crystals appear to light up. Phase contrast enhances the outline of a structure and can be particularly useful to visualize dysmorphic red blood cells [11]. Routine use of stains for urinary sediment, once taboo, is now in vogue. The Sternheimer-Malbin stain (crystal-violet and safranin) can help distinguish nucleated cells from other structures and Sudan stain can help identify lipids (though maintaining stain integrity can be a challenge if it is used infrequently). In contrast, the use of Wright or Hansel stain for urine eosinophils is no longer supported, since the finding of urine eosinophils is neither sensitive nor specific for acute interstitial nephritis [12]. Finally, it is worth noting that additional techniques employed with provider performed microscopy has implications for maintaining provider competency and compliance with the Clinical Laboratory Improvement Amendment (CLIA) [13].

The clinical laboratory, constrained by the requirements for reproducibility and efficiency can provide useful information regarding cell count as a screening tool to prompt a more in-depth investigation of the urinary sediment. Next, provider performed microscopy with time and improved microscopy techniques can provide a range of exciting images to improve clinical care.

Author statement

Melanie P. Hoenig-conceptualized the report, prepared the initial draft and revision. Jose D. Mena-contributed to the initial draft. Stewart H Lecker-contributed to the draft and created the figure.

Declaration of competing interest

The authors report no conflicts of interest.

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