Traditional Urinary Biomarkers in the Assessment of Hospital-Acquired AKI

Mark A. Perazella and Steven G. Coca

Summary

Traditional biomarkers, such as urine chemistries and urine microscopic elements, are used in the diagnosis and care of patients with AKI. Urine chemistries, such as fractional excretion of sodium and fractional excretion of urea, are useful for differentiating prerenal AKI from acute tubular necrosis only in select patients. Urine microscopy using a quantitative evaluation of the urine sediment for renal tubular epithelial cells, renal tubular epithelial cell casts, and granular casts has recently been shown to differentiate prerenal AKI from acute tubular necrosis and also provide prognostic information. Urine microscopy has also been noted to compare favorably with new urine biomarkers for diagnosis and prognosis of AKI. Thus, current information on urine diagnostics suggests that urine chemistries have a limited role in differential diagnosis of AKI, whereas urine microscopy and new urine biomarkers may be used together to differentiate prerenal AKI from acute tubular necrosis and predict such outcomes as worsened AKI, acute dialysis, and death.


“...the ghosts of dead patients that haunt us do not ask why we did not employ the latest fad of clinical investigation. They ask us, why did you not test my urine?”

—Sir Robert Grieve Hutchison (1871–1960)

Introduction

AKI occurs commonly in the hospital, and its incidence continues to increase (1). Of note, AKI is associated with multiple adverse outcomes, including incident and progressive CKD, ESRD, and death (2). In the hospital, most AKI cases are due to prerenal AKI or acute tubular necrosis (ATN), with a small contribution from acute glomerular, vascular, and interstitial diseases and urinary obstruction. We focus on the two most common causes of AKI.

Evaluation of patients with AKI has become more standardized through the use of such definitions as the Risk-Injury-Failure-Loss-End Stage (RIFLE) and Acute Kidney Injury Network (AKIN) criteria to diagnose and classify this entity (3,4). These criteria, however, do not permit differentiation of the various types of AKI, including prerenal and ATN, which require inhomogeneous management. This is particularly relevant because excessive fluid repletion in patients with ATN may be associated with untoward outcomes. Thus, clinicians depend on clinical tools such as history, examination, ultrasonography, and certain laboratory data to make this distinction. For the most part, the laboratory tests used to differentiate these two common causes of hospital-acquired AKI are urinary tests: urine chemistries and urinalysis with urine microscopy.

Urine Chemistries

Fractional Excretion of Sodium

Fractional excretion of sodium (FeNa) is calculated as follows:

$$\frac{\frac{U}{P}Na}{\frac{U}{P}Creatinine \times 100}$$

where U indicates urine and P indicates plasma. It was one of the first urine chemistries used to differentiate prerenal AKI from ATN. It is based on the premise that intact tubules will reabsorb sodium in the pre-renal setting whereas injured tubules occurring with ATN will not. In 1976, Espinel tested this hypothesis in 17 highly selected patients with oliguric AKI (5). In contrast to urine sodium, FeNa tested well in this group: 1% for prerenal and 3% for ATN. It is notable that patients with CKD, glucosuria, bicarbonaturia, and other comorbid conditions were excluded. A follow-up study in 87 patients with AKI and prerenal, oliguric ATN, and nonoliguric ATN noted a separation of prerenal AKI from both types of ATN (6). However, many patients in the ATN groups, in particular nonoliguric ATN, had FeNa levels >1%. Patients with obstruction had high FeNa, whereas those with acute GN had a low FeNa level. Subsequently, numerous authors described various forms of ATN with FeNa <1% and prerenal AKI with FeNa >1% (8). One of the major reasons for reduced FeNa utility relates to the use of this test in disease states (Table 1) not included in the initial studies. Such
Table 1. Limitations of fractional excretion of sodium

<table>
<thead>
<tr>
<th>Scenarios with FeNa &lt; 1%</th>
<th>Scenarios with FeNa &gt; 2%</th>
</tr>
</thead>
<tbody>
<tr>
<td>normal kidney function with low or moderate salt intake</td>
<td>normal kidney function with high salt intake or IV saline</td>
</tr>
<tr>
<td>acute GN</td>
<td>late urinary obstruction</td>
</tr>
<tr>
<td>early AIN</td>
<td>late AIN</td>
</tr>
<tr>
<td>acute urinary obstruction</td>
<td>glucosuria</td>
</tr>
<tr>
<td>transplant rejection</td>
<td>bicarbonaturia</td>
</tr>
<tr>
<td>FeNa &lt; 1% despite ATN</td>
<td>FeNa &gt; 2% despite prerenal AKI</td>
</tr>
<tr>
<td>AKI with liver failure or CHF</td>
<td>use of diuretics</td>
</tr>
<tr>
<td>sepsis-associated AKI</td>
<td>CKD</td>
</tr>
<tr>
<td>radiocast nephropathy</td>
<td>FeNa after IVF therapy</td>
</tr>
<tr>
<td>nonoliguric ATN</td>
<td>glucosuria</td>
</tr>
<tr>
<td>myoglobinuric ATN</td>
<td>bicarbonaturia</td>
</tr>
<tr>
<td>hemoglobinuric ATN</td>
<td>salt-wasting disorders</td>
</tr>
</tbody>
</table>

FeNA, fractional excretion of sodium; AIN, acute interstitial nephritis; ATN, acute tubular necrosis; CHF, congestive heart failure; IV, intravenous; IVF, intravenous fluid.

Diseases disturb the typical tubular response to sodium reabsorption and make FeNa poorly reflective of the actual cause of AKI.

**Fractional Excretion of Urea**

Fractional excretion of urea (FeUrea) is calculated as follows:

\[
\text{FeUrea} = \left( \frac{U}{P} \right)_{\text{urea}} \left( \frac{U}{P} \right)_{\text{creatinine}} \times 100.
\]

It is based on the same premise as FeNa except that urea reabsorption is less affected by loop and thiazide diuretics, which act distally to the proximal tubule (the major site of urea reabsorption). FeUrea <35% reflects prerenal AKI, whereas FeUrea >50% reflects loss of tubular function from ATN. In 1992, Kaplan and Kohn noted a discordance between FeNa >2% and FeUrea <35% in 6 patients with prerenal AKI (9). Retrospective chart review by these authors noted a similar discordance in 20 prerenal patients. Ten years later, Carvounis and colleagues tested the utility of FeUrea in 102 patients with prerenal AKI (n=50 without diuretic; n=27 with diuretic) and ATN (n=25) of various causes (10). Diagnosis was based on clinical measures, urine microscopy, urine chemistries, and “response to therapy.” FeUrea <35% tested very well, with a sensitivity of 90% and a specificity of 96%, for the diagnosis of prerenal AKI. Subsequent study of FeUrea had less favorable results. Pépin and colleagues tested FeUrea in 99 patients with prerenal AKI (n=23 with no diuretic; n=43 with diuretic) or ATN (n=12 with no diuretic; n=21 with diuretic) and, in contrast to the previous study, found the test unhelpful and slightly worse than FeNa (11). FeUrea <35% had a sensitivity of 68% and a specificity of 48% for prerenal AKI. Results were no better when stratified by diuretic use. Possible explanations for the difference in testing results between these two studies exist. For example, different definitions of prerenal and ATN were used. Moreover, in Pépin and colleagues’ study, patients were older and sicker, with more comorbid conditions (such as CKD and diabetes mellitus) and need for intensive care unit care. Pépin and coworkers’ study had more patients who were nonoliguric and had an increased time from AKI diagnosis to specimen collection. This last point is particularly relevant because it could have allowed interventions such as intravenous fluids to be administered before measurement of FeUrea.

Diskin and colleagues examined the utility of FeUrea (<40% cutoff) in 100 consecutive hospitalized patients with oliguric AKI (<600 ml/d) due to prerenal AKI (n=80) or intrinsic AKI (12). FeUrea <40% tested very well in detecting prerenal AKI (98% overall accuracy [78 of 80 patients]), even in the presence of diuretics (correct in 54 of 56 patients). Although FeUrea performed well in oliguric prerenal AKI, it is unclear whether the study included patients with underlying CKD, diabetes mellitus with glucosuria, or bicarbonaturia, which can reduce test accuracy.

Thus, urine chemistries such as FeNa and FeUrea appear to have a limited role in differentiating prerenal AKI from ATN in many patients with hospital-acquired AKI. As such, these tests should be used only in specific patients and in certain clinical scenarios.

**Urine Microscopy**

A time-honored laboratory test used to evaluate AKI is urine microscopy. For example, visualization of red blood cell casts in the urine sediment is fairly definitive for GN. In addition, numerous textbooks and review articles posit that the presence of renal tubular epithelial cells (RTECs), coarse granular casts, and RTEC casts in the urine is evidence for ATN, whereas bland sediment and hyaline casts are consistent with prerenal AKI. This is a logical claim because an ischemic or nephrotoxic insult causes tubular injury, with resulting apoptosis or necrosis of RTECs. The RTECs are shed into tubular lumens, where they are excreted free or as RTEC casts or granular casts (Figure 1) that can be examined in a fresh urine sediment. An important concept is that prerenal AKI and ATN are a spectrum and may sometimes coexist. As such, the urine sediment would be expected to have more cells and casts with severe ATN as
compared with prerenal AKI with patchy tubular injury; thus, it seems logical to assess the urine findings quantitatively. Most nephrologists prescribe to this way of thinking, but until recently the literature confirming this practice was relatively limited.

**Differential Diagnosis**

Small studies have shown the utility of urine microscopy in differentiating prerenal AKI from ATN. In 1991, Graber and coworkers focused on cells and casts present in the urine of 21 patients with hospital-acquired ATN. Urine microscopy demonstrated classic RTECs in 76% of patients, granular casts in 62%, and atypical RTECs with cytoplasmic vesicles in 76%, suggesting that a thorough urine sediment examination could confirm a diagnosis of ATN in approximately three quarters of patients. Perazella and colleagues examined the utility of urine microscopy and a urine sediment score (based on RTECs and granular casts) in 231 patients with hospital-acquired AKI due to prerenal AKI or ATN. The likelihood ratios for ATN increased in a dose-dependent fashion as the number of granular casts or RTECs increased and declined for prerenal AKI (Table 2). In patients with an initial diagnosis of ATN (before urine microscopy), the presence of granular casts or urine sediment score ≥2 had a positive predictive value of 100% for a final diagnosis of ATN. In patients with an initial diagnosis of prerenal AKI, the lack of granular casts or a urine sediment score of 1 had a negative predictive value of 91% for a final diagnosis of prerenal AKI. Thus, critical performance of urine microscopy appears to be useful to differentiate the most common causes of hospital-acquired AKI.

**Prognosis**

Although AKI differentiation is important to guide appropriate therapy, the current era of assessment of the utility of diagnostic tests demands that important clinical end points are measured. In AKI, worsening of kidney function, requirement for dialysis, and death are such end points. Marcussen and colleagues undertook such a study in 51 patients with hospital-acquired AKI who had prerenal AKI, "non-ATN" AKI, or ATN. They found that the number of urinary cells and granular casts had a positive correlation with the magnitude of increase in serum creatinine (i.e., worsening AKI). In addition, dialysis-requiring patients had more RTECs and granular casts than patients who did not require dialysis. In 2008, Chawla and colleagues demonstrated that an "AKI cast scoring index" was useful in predicting severity and nonrecovery from AKI. Their score, based on RTEC casts and granular casts, was derived from 30 patients with ATN by three blinded readers. A higher cast score (2.55 versus 1.57; \(P=0.04\)) was associated with AKI nonrecovery compared with AKI recovery (Table 3).

In 2010, Perazella and coworkers used urine microscopy and a modified urine sediment score (Figure 2) to evaluate the outcome of worsening AKI in 197 patients with AKI that was defined by the AKIN criteria and was due to prerenal AKI or ATN. The composite outcome of "worsening AKI" consisted of higher AKIN stage, dialysis requirement, and death. With use of a urine sediment score based on granular casts and RTECs, increasing urine score was associated with an increasing adjusted relative risk for worsening AKI (score, 0 versus ≥3; relative risk, 7.3 [95% confidence interval, 3.8–9.6]). The urine sediment score remained predictive for worsening AKI for all the individual outcome components (Table 3 and Figure 3). Thus, urine microscopy appears to have utility not only in differentiating AKI but also in predicting severity of AKI and death.

**Urine Diagnostics in the 21st Century: Requiem for Traditional Urine Biomarkers?**

Urine diagnostics in the 21st century have focused on the development and evaluation of novel biomarkers for AKI. These new urinary proteins were discovered in animals exposed to ischemia reperfusion, nephrotoxins, and other forms of kidney injury. Multiple studies have shown them to also increase in various forms of human AKI. Studies of these novel biomarkers have examined their utility not only in the differential diagnosis of AKI but also in identifying early AKI and predicting clinical outcomes.
With the rapid development of novel urinary biomarkers as a point-of-care test for AKI, one is left to ponder whether traditional biomarkers such as urine chemistries and urine microscopy will become outdated. As reviewed, FeNa and FeUrea are easy to obtain but are useful for differentiating AKI only in select groups of patients. Urine microscopy is inexpensive and readily available and maintains good performance in differential diagnosis and predicting clinical outcomes, but it requires training and experience. In addition, it is time consuming—it requires obtaining a fresh urine specimen, centrifuging the urine and preparing the slide, and viewing numerous fields under the microscope. In addition, the Clinical Laboratory Improvement Amendment limits the clinician’s ability to perform this test outside the central laboratory and requires other forms of certification, such as for Provider-Performed Microscopy. Thus, it appears that the field is ripe for a replacement test, such as the novel urine biomarkers. However, before traditional markers are deemed obsolete, a comparison of their performance

Table 2. Likelihood ratios for prerenal AKI and acute tubular necrosis based on urine microscopy (14)

<table>
<thead>
<tr>
<th>Urine Findings</th>
<th>ATN</th>
<th>Prerenal AKI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Granular casts/LPF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>23</td>
<td>84</td>
</tr>
<tr>
<td>1–5</td>
<td>73</td>
<td>21</td>
</tr>
<tr>
<td>6–10</td>
<td>23</td>
<td>2</td>
</tr>
<tr>
<td>&gt;10</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>total</td>
<td>125</td>
<td>106</td>
</tr>
<tr>
<td>RTE cells/HPF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>75</td>
<td>88</td>
</tr>
<tr>
<td>1–5</td>
<td>38</td>
<td>18</td>
</tr>
<tr>
<td>6–20</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>&gt;20</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>total</td>
<td>125</td>
<td>106</td>
</tr>
</tbody>
</table>

ATN, acute tubular necrosis; LPF, low-power field; RTE, renal tubular epithelial; HPF, high-power field.

Table 3. Studies evaluating urine microscopy and prognosis of AKI

<table>
<thead>
<tr>
<th>Study Year (Reference)</th>
<th>Population</th>
<th>Patients (n)</th>
<th>Scoring System</th>
<th>Outcomes</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chawla et al., 2008 (16)</td>
<td>AKI on renal consult service</td>
<td>18</td>
<td>Grade 1–4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Renal nonrecovery</td>
<td>AUC, 0.79</td>
</tr>
<tr>
<td>Perazella et al., 2010 (17)</td>
<td>AKI on renal consult service</td>
<td>197</td>
<td>Score 0 to ≥3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Worsened AKI (increase in AKIN stage, RRT, or death)</td>
<td>AUC, 0.75</td>
</tr>
<tr>
<td>Bagshaw et al., 2011 (23)</td>
<td>ICU patients with AKI</td>
<td>83</td>
<td>Score 0 to ≥3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>A) Worsened AKI B) RRT/death</td>
<td>Score 1–2: OR, 5.6</td>
</tr>
<tr>
<td>Hall et al., 2011 (24)</td>
<td>AKI ≥ stage 1</td>
<td>249</td>
<td>Score 0 to ≥3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Worsened AKI (increase in AKIN stage, RRT, or death)</td>
<td>AUC, 0.66; NRI, 24%</td>
</tr>
</tbody>
</table>

AUC, area under the curve; AKIN, Acute Kidney Injury Network; RRT, renal replacement therapy; RR, relative risk; ICU, intensive care unit; OR, odds ratio; NRI, net reclassification index.

<sup>a</sup>Grade 1: none (no casts or renal tubule epithelial [RTE] cells); grade 2: at least 1 cast or RTE cell seen but <10% of low-power field (LPFs); grade 3: many casts and RTE cells seen on >10% but <90% of LPFs; grade 4: sheets of muddy brown casts, casts, and RTE cells seen on >90% of LPFs.

<sup>b</sup>0 points: 0 casts or 0 RTE cells; 1 point each: 1–5 casts per LPF or 1–5 RTE cells per high-power field (HPF); 2 points each: ≥6 casts per LPF or ≥6 RTE cells per HPF.

<sup>c</sup>0 points: 0 casts or 0 RTE cells; 1 point each: 1 cast or 1 RTE cell per HPF 2 points each: 2–4 casts or RTE cells per HPF; 3 points each: ≥5 casts or RTE cells per HPF.
with that of new biomarkers is required to provide a definitive answer.

Traditional versus Novel Urine Biomarkers

Koyner and colleagues noted the lack of utility of FeNa and FeUrea in early detection and clinical prognosis of AKI in patients undergoing cardiac surgery compared with several novel biomarkers (22). The first study to compare traditional urinary biomarkers (including urine microscopy) with novel biomarkers was undertaken in 83 intensive care unit patients with AKI by Bagshaw and colleagues (23). Their prospective, two-center cohort study examined the utility of a urine microscopy score (UMS), urine chemistries, and plasma/urine neutrophil gelatinase-associated lipocalin (NGAL) in predicting worsening kidney function, dialysis need, and death in septic and nonseptic patients with AKI. The UMS was higher in septic patients with AKI, correlated with urine NGAL ($r=0.41$; $P=0.012$), and predicted worsening AKI (UMS, 0 versus $\geq 3$; adjusted odds ratio, 8.0) by increased RIFLE criteria. UMS was associated with greater likelihood of dialysis requirement and crude hospital death. A UMS $\geq 3$ had the following characteristics for detecting "worsening AKI": sensitivity, 0.67; specificity, 0.95; positive predictive value, 0.80; and negative predictive value, 0.91. Urine chemistries (urine sodium, FeNa, FeUrea) were not associated with the clinical outcomes. Thus, urine microscopy and urine NGAL correlate fairly well and are complementary in predicting worsening AKI in intensive care unit patients (Table 3).

Most recently, Hall and coworkers undertook a prospective cohort study to evaluate traditional urine biomarkers and novel urine biomarkers in hospitalized patients who developed early AKI according to the AKIN criteria (24). They tested the utility of these biomarkers in predicting several clinical outcomes as well as differentiating various forms of AKI. After exclusion, 249 patients were enrolled on the first day of meeting AKI criteria, more than half were older than 65 years of age, and nearly 50% were in the intensive care unit; the mean baseline GFR was $69\pm 30$ ml/min per 1.73 m$^2$. The causes of AKI were as follows: prerenal AKI (66%), ATN (20%), and “other” (14%). Seventy-two patients (29%) met the primary composite outcome of “worsened AKI or in-hospital death.” The adjusted risk for the primary outcome was approximately threefold higher in those with upper than in those with lower values of urine NGAL, kidney injury molecule-1, IL-18, and microscopy score (Figure 4). Secondary outcomes, such as higher AKI stage, dialysis requirement, nephrology consultation, and death, were associated with higher urine biomarker quartiles or microscopy score. FeNa and FeUrea were not useful for differential diagnosis or predicting outcomes. Importantly, risk classification of AKI (determined by the net reclassification index and integrated discrimination improvement) was significantly improved after the novel biomarkers or urine microscopy measures were added to standard clinical variables. These data suggest that novel urine biomarkers and urine microscopy are useful to differentiate early hospital-acquired AKI and improve upon the baseline clinical determination of prognosis (Table 3).

Moving Forward

So what guidance can we recommend to clinicians who care for patients who develop AKI while hospitalized? For diagnosis, FeNa and FeUrea must be used in an evidence-based fashion—knowing the limitations of the tests and the clinical scenarios in which the tests operate well and where they fail. With regard to urine microscopy, using a quantitative approach to urine sediment findings (numbers of cells or casts per low- or high-power field) will improve diagnostic accuracy, not only for glomerular diseases but also for prerenal AKI and ATN. Sometimes combining urine chemistries and quantitative urine microscopy will provide better diagnosis and distinction of prerenal AKI from ATN. Moreover, urine microscopy also adds prognostic information, which can help the clinician plan ahead for the patient (dialysis preparation, family counseling). Although these traditional tests are
inexpensive and readily available, their limitations may provide a niche for novel biomarkers, which may provide useful point-of-care information about AKI diagnosis and prognosis in the future.

Because AKI outcomes have not measurably improved over the past several years, and the number of CKD cases after AKI may exceed 100,000 per year (25–28), we must intensify efforts to improve outcomes. To accomplish this, data from observational studies should be incorporated into the design of randomized controlled trials of AKI therapies. Because therapies are more likely to demonstrate benefit only for patients who experience persistent AKI (i.e., ATN), urine microscopy should be used as an inclusion criterion for trial enrollment. This would allow only patients who have a high likelihood of progressing to more severe AKI, such as those with ATN, to enter studies. Perhaps this approach will permit the nephrology community to identify beneficial therapies for AKI.

In conclusion, on the basis of the current information on urine diagnostics, it appears that urine microscopy and new urine biomarkers may be used together to inform on early AKI, differentiate prerenal AKI from ATN, and predict such outcomes as worsened AKI, dialysis requirement, and death. Urine chemistries may provide some insight into differential diagnosis when used in select patients but have little role in most hospitalized patients with AKI. Figure 5 shows one possible approach for urine diagnostics in patients with hospital-acquired AKI.

**Figure 3.** Urine sediment score and individual components of the composite outcome. AKIN, Acute Kidney Injury Network (17).

**Figure 4.** Urine biomarker groups (neutrophil gelatinase-associated lipocalin, IL-18, kidney injury molecule (KIM-1) in quartiles and urine microscopy score of 0, 1, 2, ≥3) and primary outcome occurrence (worsened AKI or in-hospital death) (24).
Figure 5. | Potential approach to AKI using traditional and novel biomarkers. FeNa, fractional excretion of sodium; FeUrea, fractional excretion of urea; RRT, renal replacement therapy.

References


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