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Urine Microscopy Findings Predict Outcomes In Hospitalized Patients With Acute Kidney Injury

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ABSTRACT

URINE MICROSCOPY FINDINGS PREDICT OUTCOMES IN HOSPITALIZED PATIENTS WITH ACUTE KIDNEY INJURY

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Though urine microscopy has long been highly regarded by nephrologists as an essential diagnostic tool, its potential utility in predicting outcome in acute kidney injury (AKI) warrants further exploration. In this study, urine sediment microscopy was performed on 165 hospitalized patients on the first day of their clinical AKI diagnosis to determine whether microscopy findings early in the course of this disease correlate with "worsening," a composite of increasing AKI stage and in-hospital mortality. Microscopy findings were recorded as individual cells and casts along with a microscopy score derived from renal tubular epithelial (RTE) cells and granular casts. Our data suggest that both increasing numbers of granular casts and a higher microscopy score are predictive of overall worsening ($p = 0.027$ and $p = 0.046$, respectively), but other microscopy features such as the number of RTE cells are not. These data demonstrate that urine microscopy even at the time of initial diagnosis can be predictive of eventual outcome, potentially serving as a useful adjunct to clinical and other biochemical data in the study of AKI.

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INTRODUCTION

Uroscopy: Early Physicians and the Art of Looking at Urine

As urine was one of the most readily available and easily accessible bodily fluids, early physicians and scientists focused their efforts on gleaning all the information they could about the mysterious workings of the human body by noting the physical characteristics of urine, a process known as uroscopy. Though ancient Sanskrit and Sumerian texts indicate that several ancient civilizations were making important observations about the properties of urine, it is the Greeks and Hippocrates, in particular, who are often credited with being the first uroscopists (1). Hippocrates' treatise, *Prognostics*, became one of the most widely propagated texts on the subject of uroscopy, delineating the properties of urine that would be studied for many centuries to follow including its color (white, red, black), consistency (thin, thick, watery, clear, cloudy), sediment (smooth, leafy, farinaceous, absent), odor (fetid), and volume (deficient) (2). Hippocrates' initial observations were later expanded by the Roman physician Galen who believed that the study of the urine was an important means of determining the relative balance of the four humors (blood, phlegm, yellow and black bile), which he hypothesized were the determinants of disease (2).

By the 13th century, uroscopy was a well-established field and efforts were being made to improve its scientific accuracy. In order to view the urine more clearly and to more accurately describe its properties, physicians debated the appropriate characteristics of the vessel in which urine should be stored. Since it was believed that urine would behave most physiologically in a receptacle that was bladder-shaped, the matula was born, a clear glass vessel with a rounded-bottom that was held up to the light for best

inspection of the urine held within (1). Uroscopy had become so entrenched in the practice of medicine that soon after the matula was introduced into common practice, it quickly became the symbol of the Medieval physician and was widely depicted in art of the time (2). The matula itself would undergo many changes throughout the centuries, gradually acquiring increasing layers of complexity. In fact, by the 16th century, the matula had become human-body shaped and had been subdivided into twenty-four levels in an effort to improve the precision of diagnosis. It was thought that the urine vapors would congregate into the level corresponding to the diseased body part (2).

In fact, so much store was set in the powerful diagnostic value of uroscopy that by the 14th century, it quickly became subject to much abuse. Charlatans began dabbling in the art of uroscopy, recognizing its moneymaking potential. These unscrupulous quacks would offer to analyze the urine of their unsuspecting public, come to an outrageous diagnosis, and offer their now terrified 'patient' an incredibly expensive 'cure'(2). Ironically, physicians were in no small part contributing to this behavior. As their confidence in their uroscopy skills grew, physicians began claiming that physical examination of the patient had been rendered unnecessary as all necessary diagnostic information could be obtained by examination of the urine (2). Textbooks on uroscopy became available to the general public so that people began diagnosing and treating themselves after observing the characteristics of their own urine (1). By the late 16th century, the excesses of the field had grown to such a degree that 'uromancy' was born which focused on divining the future from examination of the urine (1).

In response to these excesses, the 16th century saw a backlash against the practice of examining urine in diagnostic medicine. Author Thomas Brian wrote *Pisse Prophet* in

1637, a scathing attack on uroscopy which quickly made the matula and physicians who used it the subject of much ridicule and scorn (1). Thomas Linacre, the founding president of the College of Physicians in England, instituted a statute preventing physicians from continuing the practice of examining urine in lieu of examining their patients, and members of the college began producing works that favored a more measured view of the benefits and limits of uroscopy (2). The Enlightenment's focus on rationality and science helped shift the focus of uroscopy from the observable physical characteristics of urine to a more sophisticated biochemical analysis of urine's components, a shift that would be aided immensely by the invention of the microscope.

A Brief History of Urine Microscopy

Though the first observation of the urine with the aid of a microscope was likely performed in the 17th century, technology at the time was rudimentary at best and so the analyses possible at that time were also. Early uses of microscopy focused on the study of kidney stones as this was a disease of the rich and one in which microscopic analysis of the urine for stone precursors was thought to be quite promising (3). Urine microscopy remained quite limited until the first half of the 19th century when advances in the technology and availability of microscopes made it possible for the field of urine microscopy to grow and begin to contribute to our understanding of renal pathology.

Three Frenchmen share the distinction of being some of the earliest physicians to perform sophisticated analyses of urine via microscopy: Pierre Rayer, a physician at l'Hôpital de la Charité in Paris, his intern, Eugène Vigla, and their contemporary and academic rival, Alfred Donné (4). Though Donné published some of the first daguerrotypes of the microscopic elements of urine, it was truly Rayer and Vigla who

more methodically described and more clearly articulated the potential clinical implications of their findings (3). Their extensive findings were initially published in the French journal *l'Expérience* in 1835 before being included in a comprehensive textbook *Traité des maladies des reins* (4). Rayer and Vigla developed the first standards of how to properly handle urine and identified important microscopic constituents of urine including crystals, squamous cells, mucus, pus, blood, lipids, sperm, yeast, and possibly even casts (4). They also began to recognize and describe the microscopic appearance of clinical syndromes, recognizing the potential utility of microscopy in clinical nephrology. For example, they noted that in acute nephritis there was often a collection of blood, mucus, squamous cells, and fibrin in the urine while in nephrotic syndrome they described thin lamellae of amorphous substance (possibly casts), mucus, lipids, blood, and uric acid crystals (5). Thus, even when in its infancy, urine microscopy was found to have observable patterns that not only correlated with clinical findings but could perhaps be instrumental in differentiating between clinical syndromes.

These early advances in France led to a wave of discovery in England and Germany, the leading scientific nations at the time. Johann Simon in Germany focused on casts, recognizing them as different in character and appearance from other cells in the urine. He and other German scientists Julius Vogel and Hermann Nasse first understood that the casts they were identifying were derived from the tubular epithelium (4). Meanwhile, in England, Golding Bird published *Urinary deposits. Their diagnosis, pathology, and therapeutical indications* in 1844, a work that expanded on many of the findings of his French colleagues and introduced microscopy to the English scientific

community (4). Bird's work was limited in that its primary focus was on crystals with only one chapter focusing on the noncrystalline elements of urine (3).

The second half of the 19th century saw further improvements in the technology of lenses, leading to improved image quality and thus, to further advances in the field of urine microscopy. The concept of staining was also being introduced to the field at this time, a key development that would allow the Italian scientist Carlo Rovida to make the observation that casts were composed of a unique protein which would only be identified almost a century later (3). Centrifugation of the urine sample prior to microscopy also became standard practice, ensuring that all the salient features of the sediment were captured and examined. By the end of the century, atlases were published around the world carefully detailing the typical urine sediment found in different renal diseases and urine microscopy was beginning to be incorporated in medical school curricula in North America, signs that urine microscopy was becoming a well-established part of clinical practice.

In the 20th century, urine microscopy as practiced today was perfected. In the 1920s the Scottish-American Thomas Addis did his famous studies on his glomerulonephritis patients, following their urines serially and noting that their sediment findings would become blander as their disease transitioned from the acute phase to the chronic. He also developed his own method of quantifying urine microscopy findings using collection of timed urine and counting chambers, a method that would eventually be named after him and which is still in use today (3). With this method, Addis was able to describe 'renal failure casts,' broad casts that were found in extremely high quantities in the urine of patients who were dying of uremia (6).

Table 1 Urinalysis findings in renal disease

Component	Clinical significance
Cellular	
Eosinophils	AIN, atheroembolic disease, pyelonephritis, glomerular disease
Granulocytes	infection, nephritis
Red blood cells (dysmorphic RBCs)	glomerular disease
RTE cells	AKI, AIN, glomerular disease
Uroepithelial cells	neoplasia, stones, UTI
Casts	
Hyaline	non-specific, normal finding
Granular	ATN
Waxy	chronic renal impairment
Fatty	nephrotic syndrome
RBC casts	glomerular disease
WBC casts	AIN, pyelonephritis, glomerular disease
RTE cell casts	ATN, AIN, nephrotic syndrome, glomerular disease
Other	
Lipids	nephrotic syndrome
Bacteria	infection
Crystals	inherited metabolic disorders, toxins

AIN = allergic interstitial nephritis; UTI = urinary tract infection
Adapted from DeLanghe 2000 (7) and Fogazzi 2011 (8)

The latter half of the 20th century saw the introduction of new types of microscopy to the study of the urine, including immunofluorescence, transmission electron microscopy and phase contrast, the first of which would be instrumental in the identification of Tamm-Horsfall glycoprotein in the matrix of casts (9). Phase contrast microscopy was quickly recognized as far superior to its predecessor, as it allowed for more precise visualization of structures in the sediment (10). In the 1980s, interest in urine microscopy was renewed when Fairley and colleagues noted that the morphology of RBCs could be a useful key to distinguishing glomerular from non-glomerular hematuria (11).

Thus, the study of the urine, whether with the eye as in ancient times or more recently with the aid of microscopy, has always played been an important role in improving our understanding of renal diseases. As a result of the work of these pioneering scientists and physicians, sophisticated interpretation of urinalysis is now routine and serves a useful tool in the diagnosis of kidney pathology (see Table 1).

Limitations of Urine Microscopy and Future Directions

Despite its well-established place as a diagnostic tool in the field of nephrology, urine microscopy does not come without its limitations, most of which have been well-documented (7). Primary among these limitations is the considerable variability inherent in the technique in everything from how samples are prepared to who or what is analyzing them. A study by Winkel et al in 1978 methodically explored many of these sources of error, concluding that the greatest source of error lay in sample preparation, especially in centrifugation (12, 13).

In addition to questions of how best to prepare and handle samples, there has been some debate as to the best means of quantifying urine sediment findings. The typical method involves counting the number of cells per high power field (HPF) which is a lower cost and less labor intensive method than chamber counting, a more quantitative technique which allows for identifying cellular elements per mL of sample. One study by Kesson et al showed that in a series of 88 duplicate urines, abnormal numbers of casts or white or red blood-cells were detected by the quantitative method in 74% of patients with abnormal renal function but in only 36% by the HPF method (14). Though it is known that urine sediment findings are imprecisely recorded with the HPF method, current guidelines still recommend this method over chamber counting because its ease of use and low cost make it much more feasible in daily practice (15).

Another much noted source of considerable error is of course interobserver variability. A study by Tsai et al found that this variability could potentially have a significant impact on a physician's ability to make the proper diagnosis. Urine samples were taken from 26 patients diagnosed with acute kidney injury (AKI) who were seen by

the nephrology consultation service. These samples were independently analyzed by a nephrologist (nephrologist A) and by the hospital laboratory technicians. Nephrologist A correctly diagnosed the cause of AKI in 24 patients (92%) after his examination of the urine while nephrologist B, given only the laboratory UA report, was correct only 5 times (19%), improving to 69% (18 patients) after reviewing nephrologist A's UA report. Both nephrologists were blinded to the patient's clinical history. Significant differences existed between nephrologist A's microscopy report compared with the clinical laboratory's report with nephrologist A reporting a higher number of RTE cells ($P < 0.0001$), granular casts ($P = 0.0017$), hyaline casts ($P = 0.0233$), and RTE casts ($P = 0.0008$). There were no significant differences in the reporting of other urinary sediments, including WBCs, WBC casts, RBCs, RBC casts, transitional epithelial cells, oval fat bodies, bacteria, yeast, and crystals (16). These findings argue for the importance of relying on trained observers in analyzing urine microscopy. However, as shown recent study by Wald et al., interobserver variability can be significant even amongst highly trained nephrologists. Ten nephrologists of different levels of experience were asked to identify structures in the microscopy images of 86 patients who had been seen by the nephrology service. Using the κ statistic to determine the level of agreement, they found that squamous epithelial cells ($\kappa = 0.54$) and hyaline casts ($\kappa = 0.52$) had the highest levels of interobserver agreement, while identification of transitional epithelial cells ($\kappa = 0.14$) and fatty casts ($\kappa = 0.06$) had the lowest levels. They also measured overall agreement in the presence or absence of a structure, finding that complete agreement for most types of casts was generally better (59%-79%) than for most cellular elements (31%-39%) with the notable exception of coarse and fine granular casts (32%

and 37%, respectively) (17). Thus, even in identification of simple cellular elements, measurable and significant variability can exist between trained observers.

Different types of microscopy also offer their own advantages and disadvantages. Though traditional bright field microscopy is easiest and often most commonly used, its relatively poor visualization capabilities make it difficult to distinguish bacteria from amorphous debris or leukocytes from small tubular cells (10). In fact, all of the most recent urinalysis guidelines put out by Finnish, Japanese, and European societies note to the inadequacy of bright field microscopy even if accompanied by supravital staining, recommending phase contrast microscopy instead (10, 15).

In an effort to minimize these potential sources of error and to improve standardization and reliability of urine microcopy results, there has been a move towards automated microscopy over manual microscopy in the field. Several studies have shown that the automated systems currently in use (ie the Sysmex UF-100, sediMAX, and the Iris iQ200) are more precise than manual techniques in identifying cellular elements though not casts (18-21). Even as technology evolves and automated identification of casts improves, nephrologists undoubtedly will continue to rely on a combination of automated and manual techniques to assist them in their study of renal diseases.

Acute Kidney Injury: Definition, Pathophysiology and Clinical Features

Acute kidney injury (AKI), formerly known as acute renal failure, affects somewhere between 2-18% of all hospitalized patients and as many as 25-30% of patients in the ICU (22). Though it has been recognized as a disease entity since World War II, there had been as many as 35 different definitions of the disease in the literature until 2002 when the Acute Dialysis Quality Initiative (ADQI) sought to establish a

consensus definition to facilitate the production of more precise and accurate research on the subject (23). They proposed the Risk, Injury and Failure, Loss of function and End-stage kidney disease (RIFLE) staging system which defined three stages of severity of AKI (risk, injury, failure) based on serum creatinine (Scr) and urine output criteria and included two outcome criteria (loss and end-stage renal disease) based on duration of RRT therapy (22) (see Table 2). Subsequent studies have indicated that even more modest changes in creatinine could lead to significant morbidity, prompting a re-evaluation of the definition and staging system in 2005 by the Acute Kidney Injury Network (AKIN) (24-26) (see Table 2). Though some recent studies have suggested that the new criteria have failed to show any improvement in predicting outcomes over the RIFLE criteria, the potential advantages of this new staging system have yet to be fully explored (27, 28).

Table 2 RIFLE and AKIN staging systems

	Criteria		
	Serum creatinine	**Urine output**	**Drop in GFR[A]**
RIFLE			
Risk	↑SCr ≥ 1.5 * baseline[B]	<0.5ml/kg/h ≥ 6h	≥25%
Injury	↑SCr ≥ 2.0 * baseline	<0.5ml/kg/h ≥ 12h	≥50%
Failure	↑SCr ≥ 3.0 * baseline or ↑SCr ≥ 0.5mg/dL from baseline or SCr ≥ 4.0mg/dL	<0.3ml/kg/h ≥ 24h or anuria ≥ 12h	≥75%
Loss	Loss of kidney function >4wks		
ESRD	Loss of kidney function >3mos		
AKIN			
Stage 1	↑SCr ≥ 1.5 * baseline[C] or ↑SCr ≥ 0.3mg/dL from baseline	<0.5ml/kg/h ≥ 6h	
Stage 2	↑SCr ≥ 2.0 * baseline	<0.5ml/kg/h ≥ 12h	
Stage 3	↑SCr ≥ 3.0 * baseline or ↑SCr ≥ 0.5mg/dL from baseline or SCr ≥ 4.0mg/dL or initiation of RRT	<0.3ml/kg/h ≥ 24h or anuria ≥ 12h	

[A] Only one of the three RIFLE criteria has to be fulfilled to qualify for a specific stage
[B] Baseline SCr is considered to be within one week for RIFLE
[C] Baseline SCr is considered to be within 48 hours for AKIN
AKIN = Acute Kidney Injury Network; ESRD = End-stage renal disease; RIFLE = Risk, Injury, and Failure, Loss of function and ESRD; RRT = renal replacement therapy
Table adapted from Lines 2009 (21)

The most common cause of AKI is ischemic injury, the pathogenesis of which has been clearly elucidated (29, 30). The renal tubular epithelial cells of the kidney are susceptible to injury due to their blood supply located in the medulla, an area that is particularly prone to ischemia. Once ischemic injury occurs, the cytoskeletal architecture of the renal tubular epithelial cells is disrupted, leading to a loss of cellular polarity and the shedding of the proximal tubule's brush border. During this initiation phase of AKI, though intracellular ATP depletion is severe, the injury to the endothelium remains sublethal and reversible, allowing for a complete recovery if the instigating insult is removed. Tamm-Horsfall protein, normally secreted by the thick ascending limb as a monomer, becomes a gel-like polymer in the high sodium environment of the distal tubule where it can combine with the debris of injured cells and brush border membranes to form casts (30). Casts and sloughed off cells obstruct the lumen, contributing to the kidney's declining glomerular filtration rate (GFR).

During the extension phase of the injury, blood flow returning to the renal cortex leads to reperfusion-related cell death while continued vasoconstriction in the medulla contributes to further RTE cell death (30). Leukocytes are recruited to the site of injury and produce inflammatory mediators and reactive oxygen species that worsen the cellular damage. RTE cells also contribute to this deleterious response by secreting chemokines and cytokines that perpetuate the cycle of inflammation.

During the maintenance phase, parenchymal injury is established and GFR reaches its nadir (30). At this time, a balance is maintained between continued cell death and incipient attempts at cellular regeneration. The process of cellular recovery involves dedifferation of viable cells followed by proliferation and eventual restoration of the

normal epithelium (30). Finally, during the recovery phase of the injury, the GFR gradually improves as the integrity of the epithelial cells is restored. Though the kidney has the capacity to fully and completely repair itself after sustaining this type of acute injury, a partial repair can generate a state of persistent inflammation in the kidney that can eventually manifest years later as end-stage renal disease (ESRD) (29).

The etiologies of AKI are typically divided into three categories: pre-renal, intrinsic, and post-renal. Outside of the ICU, 55-60% of AKI is pre-renal, 35-40% is intrinsic and <5% is post-renal (31). Determining the cause of AKI necessitates a diagnostic workup consisting of a history, a focused physical to assess volume status, basic laboratory tests including a BUN/Cr ratio, and imaging studies such as a renal ultrasound, if necessary. Special attention is of course paid to examination of the urine, especially to the measurement of urine electrolytes (in particular the fractional excretion of sodium or FeNa) and to the analysis of the urine sediment via microscopy. Together, these various aspects of the diagnostic workup can lead to a definitive diagnosis (see Table 3). For example, an elevated BUN/Cr ratio with a FeNa < 1% and a bland urine

Table 3 Urine studies in acute kidney injury

	Hypovolemia	ATN	AIN	Glomerulonephritis	Obstruction
Sediment	Bland	Broad, brownish granular casts	WBCs, eosinophils, cellular casts	RBCs, RBC casts	Bland or bloody
Protein	None or low	None or low	Minimal	>100mg/dL	Low
Urine Na (mEq/L)	<20	>30	>30	<20	<20 (acute) >40 (few days)
Urine Osm (mOsm/kg)	>400	<350	<350	>400	<350
FeNa (%)	<1	>1	Varies	<1	<1 (acute) >1(few days)

AIN = allergic interstitial nephritis; ATN = acute tubular necrosis; FeNa = fractional excretion of sodium; Na = Sodium; Osm = osmolality; RBC = red blood cell; WBC = white blood cell
Table adapted from Singri 2003 (32)

sediment is highly suggestive of a pre-renal etiology. If the AKI has progressed to acute tubular necrosis (ATN), the urine sediment often shows brownish granular casts with renal tubular epithelial cells. The urine sediment is also very helpful in identifying other potential cause of intrinsic renal disease such a glomerulonephritis or vasculitis in which proteinuria, dysmorphic red blood cells, and red blood cell casts may be seen (32).

Treatment of AKI is of course dependent on etiology and it remains primarily supportive. Pre-renal AKI requires appropriate volume resuscitation while other forms of AKI, including ATN, are often harmed by volume overload and require instead electrolyte repletion, avoidance of renal toxins, and RRT in cases of refractory hyperkalemia, volume overload, metabolic acidosis or uremia. Unfortunately, despite our improved understanding of AKI and advances in supportive care, mortality from the disease has remained at around 50% for the past four decades (33). Additionally, morbidity from AKI remains significant with some studies indicating as much as a three-fold greater risk for developing ESRD over the course of a decade when compared to controls (34).

One avenue towards improving outcomes in AKI lies in finding a more accurate and sensitive biomarker that will enable earlier detection of kidney injury and thus allow for administration of potentially therapeutic interventions before lasting damage occurs. Research in this field has thus far focused on several potential biomarkers of kidney disease such as cystatin C, neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), and interleukin-18 (IL-18), initially identified via microarray analysis and genomics (35). As we await large, prospective multicenter trials to determine these biomarkers' ultimate clinical utility and application, the search continues

for other laboratory tests that will aid in early detection of AKI. Also, though the AKIN stage has been shown to correlate with eventual outcomes in AKI, the need for identifying additional prognostic factors besides creatinine and urine output remains, as this would help individualize therapy in AKI, determining appropriate aggressiveness of intervention required and aiding in long-term planning of patient care (36, 37).

Urine Microscopy's Role in the Study of Acute Kidney Injury

Though tradition and consensus have suggested that urine microscopy and other urinary indices can provide important clues to the differential diagnosis of acute kidney injury, there is little evidence to support these widely held beliefs. In fact, in a systematic review of the literature of septic AKI, Bagshaw et al found that that no single measure of urinary biochemistry, derived index, or pattern on microscopy could be used reliably to diagnose AKI or predict the clinical course of AKI in these patients (38). A follow-up review by the same group then reviewed experimental studies that utilized urinary findings to monitor the progress of septic AKI in animal models, concluding that sustained oliguria, decline in glomerular filtration, and evidence of sodium retention by UNa <20 mmol/L, FeNa <1%, and Uosm >400 mosm/L appeared to be the most reliable indices of worsening AKI and that there were not enough studies that used urinary microscopy in this particular review for its utility to be determined (39). The limitations of both of these reviews were that the studies examined in each were far too heterogenous for any quantitative analysis to be performed. Also, as pointed out by the authors themselves, many of the studies reviewed had major design flaws including small study populations and retrospective design, failure to include a control group, a heterogenous patient population, failure to account for potential confounding factors, variable timing in

measurements, and inconsistent reporting of several individual urinary tests (38). Thus, the question of the utility of urinary indices, including microscopy in the study of AKI, is still very much unsettled, and several studies in the existing body of literature have attempted to prove that urine microscopy can in fact hold many useful clues to differential diagnosis and prognosis in AKI.

In an effort to firmly establish the importance of urine microscopy as a validated diagnostic tool in AKI, a study by Perazella et al in 2008 showed that urinary microscopy findings could be used in conjunction with a clinically determined pretest probability to quite definitively distinguish pre-renal AKI from ATN. In patients with a high pretest probability of ATN, any casts or renal tubular epithelial cells (RTECs) resulted in very high positive predictive value (100%) and low negative predictive value (44%) for a final diagnosis of ATN. Conversely, the negative predictive value of the absence of casts or RTECs in patients with low pretest probability of disease was 91% (40). This study helped establish likelihood ratios that would enable clinicians to calculate posttest probability of either ATN or prerenal azotemia once they have estimated the pretest probability of either cause of AKI (41). A study by Marcussen et al also examined this link between urine microscopy and the diagnosis of AKI. They found a significantly higher number of collecting duct cells and casts ($p < 0.05$ and $p < 0.03$, respectively) in the urine of 34 patients with ATN when compared to the 17 patients with non-ATN AKI (42). Similarly, a much earlier study by Gay et al analyzed the urine sediment in children who had AKI of varying etiologies and concluded that the cause of AKI could be determined by the urine sediment results, sometimes prompting an alteration of a previously made diagnosis (43).

Other studies have focused on the potential role of urine microscopy in predicting severity of AKI and thus subsequent outcomes. Schentag et al conducted daily examinations of the urine of 154 critically ill patients in order to compare cast excretion among sick patients, sick patients given aminoglycosides, and sick patients with aminoglycoside nephrotoxicity. Their results indicated that cast counts in the 30 nephrotoxic patients were 625 ± 364, significantly higher than either ICU patients who had not received aminoglycosides (44 ± 51 casts) or those who had been given aminoglycosides without a significant rise in serum creatinine (153 ± 196 casts). In some cases, cast counts began rising significantly as early as 9 days before serum creatinine first rose, indicating that urine microscopy can be an early predictor of worsening AKI (44).

In a study of patients with non-AKI renal disease, Gyory et al evaluated the correlation between the severity of pathologic changes found on renal biopsy with the number of casts excreted in the urine prior to biopsy, concluding that there was in fact a significant positive correlation ($r = 0.630$, $p < 0.01$) (45).

More recent work has further established a strong link between findings on urine microscopy and outcomes in AKI. In the previously mentioned study by Marcussen et al, they found that in addition to distinguishing ATN from other causes of AKI, urine sediment results also predicted outcomes, as patients who would eventually require dialysis consistently had higher numbers of renal cells and casts in their urine sediment (42). Chawla et al in their 2008 study established a cast scoring index (CSI) based on the numbers of granular and epithelial cell casts in the urine to improve standardization of reporting urine microscopy results and to determine whether a correlation existed

between a higher CSI and worse outcomes such as death or need for dialysis. Though the study was quite small and only included 18 patients, the patients that had worse outcomes had a higher CSI when compared with patients that recovered renal function (2.55 ± 0.93 vs. 1.57 ± 0.79, $p = 0.04$). The ROC area under the curve for CSI to diagnose worsening was 0.79 (46).

Recent work done by Perazella et al has attempted to prospectively study the relationship between urine microscopy findings at the time of nephrology consultation and clinical outcomes in AKI. Using a urinary sediment scoring system based on the number of RTECs and granular casts, they graded the urine of 197 patients with either pre-renal AKI or ATN and found that their urinary scoring system was significantly associated with increased risk of worsening AKI (adjusted relative risk: 7.3; for worsening with score of ≥ 3 *versus* score of 0) and was more predictive than AKI Network stage at the time of consultation (47).

Even electron microscopy has been used in this endeavor in studies by Mandal et al (48-50). Urinary sediments from 31 patients with oliguric acute tubular necrosis (ATN) were divided into three categories based on the severity of ultrastructural changes observed in the RTECs. Type I cells showed signs of severe and significant damage with an abundance of necrotic cells and cellular fragments, disrupted plasma membranes, dysmorphic mitochondria, and absent nuclei. Type II sediments displayed the mildest changes and were composed of a relatively homogenous population of modestly damaged RTECs. In these sediments, cellular and subcellular organelle membranes remained largely intact, and they contained normal appearing mitochondria and nuclei. Type III sediments were quite heterogenous and displayed changes intermediate to those seen in

the Type I and Type II sediments. In this study, a correlation was found between the sediment type and the severity of the clinical illness ($p < 0.05$) with only 2 out of 11 patients with Type I sediment surviving while 7 of the 8 patients with Type II sediment survived (48). Despite the potential advantages of EM, it remains prohibitively expensive to incorporate into routine practice.

Of course, some of these studies have flaws like small sample sizes, absence of control groups, and inconsistency in when the urine microscopy was performed in relation to the onset of AKI (see Table 4). Nonetheless, taken together, they support the notion that urine microscopy can play an important role in determining the cause and subsequent outcomes of AKI. Though biomarkers currently under investigation show much promise in improving our understanding of AKI, urine microscopy continues to be a simple, inexpensive, and potentially powerful way of assessing kidney dysfunction.

Table 4 Summary of studies reviewed

Author	Goals	Population	Results	Limitations
Gyory 1984	Assess whether urine sediment microscopy was predictive of histological evidence of renal disease	▪ 22 patients undergoing renal biopsy ▪ Microscopy on day of biopsy	Number of casts in sediment correlated with degree of histological change on biopsy with strong predictive value	▪ Small population ▪ No details on types of casts studied
Mandal 1985	Assess whether ultrastructural changes on TEM corresponded to outcomes in AKI	▪ TEM in 31 patients with ATN	Severity of ultrastructural changes in sediment correlated with worse outcomes	▪ Small population ▪ TEM difficult to translate to routine practice
Gay 1987	Determine utility of microscopy in differential diagnosis of AKI	▪ 31 children with AKI ▪ Microscopy within 5 days of onset	Urinary sediment examination was valuable in determining AKI diagnosis	▪ Small population ▪ No details about pattern of casts in the urine
Schentag 1987	Assess the value of quantitative cast excretion as a marker of AKI	▪ 154 ICU patients; 30 with AG toxicity ▪ Daily microscopy	▪ Cast counts were highest in those with AG toxicity ▪ In some cases, cast count rose prior to creatinine rise	▪ Daily microscopy difficult to translate to routine practice ▪ Only studied AG-related AKI
Marcussen 1995	Assess utility of	▪ 51 hospitalized	▪ Higher numbers	▪ Small population

	microscopy in differential diagnosis and outcome prediction in AKI	patients with AKI ▪ Microscopy within two weeks of peak creatinine	of cells and casts in patients with ATN than those with non-ATN AKI ▪ Higher numbers of casts in patients who needed dialysis	▪ Microscopy performed at variable times
Bagshaw 2006	Determine what indices have proven to be useful in diagnosis of AKI	Meta-analysis of 27 studies that recorded urinary indices in patients with septic AKI	▪ No measured index was consistently reliable ▪ Insufficient data to draw conclusions about microscopy	▪ Heterogeneity precluded quantitative analysis ▪ Inconsistent and variable recording of measurements across studies
Bagshaw 2007	Determine what indices could theoretically be useful in understanding AKI	Meta-analysis of 27 studies of experimental models of septic AKI	▪ Sustained oliguria, decline in GFR and evidence of Na retention were reliable indices of worsening ▪ Insufficient data to draw conclusions about microscopy	▪ Heterogeneity of studies ▪ Difficult to extrapolate from animal models to humans
Chawla 2008	▪ Devise a more uniform way to evaluate urine ▪ Determine if their score was predictive of outcome	▪ 30 patients with ATN though outcomes were only looked at in 18 ▪ Microscopy at time of consult	▪ Score was highly reproducible ▪ Patients that did not recover renal function had higher scores than those that did	▪ Small population
Perazella 2008	Assess utility of microscopy in differential diagnosis of AKI	▪ 267 hospitalized patients with AKI ▪ Microscopy at time of consult	Presence or absence of casts or RTE cells in conjunction with clinical pretest probability was highly predictive of diagnosis	▪ No biopsy-confirmed diagnoses ▪ Observers were not blinded to diagnostic impressions at time of microscopy
Perazella 2010	Determine whether urine microscopy score was predictive of outcome	▪ 197 hospitalized patients with AKI ▪ Microscopy at time of consult	▪ Higher score was associated with increased risk of worsening ▪ Score was more predictive of outcome than AKIN stage	▪ Observers were not blinded to clinical data ▪ Microscopy performed at time of consult

AG = aminoglycoside, AKI = Acute Kidney Injury, ATN = Acute Tubular Necrosis, GFR = glomerular filtration rate, ICU = Intensive Care Unit, Na = sodium, TEM = Transmission Electron Microscopy

GOALS OF THIS STUDY

Current research in the study of AKI is focused on the search for better ways of identifying incipient renal disease and for prognostic factors that can help guide treatment. Based on a small but consistent body of literature, it appears that in this endeavor, urine microscopy can be of significant utility. In particular, much work has been done in establishing a correlation between findings on urine microscopy and outcomes in AKI, but the timing of urine microscopy in these studies has been variable or late in the course of the disease (ie at the time of nephrology consultation). This study hopes to build on earlier work by prospectively analyzing urine samples collected earlier in the evolution of AKI when information about prognosis is perhaps most helpful and most needed. In particular, we attempted to ascertain whether urine microscopy results at the time of first clinical AKI diagnosis correlated with worse outcomes. If so, urine microscopy results can potentially be used to determine prognosis at a very early stage of disease and thus help direct the treatment of patients with AKI.

METHODS

Patients admitted to Yale-New Haven Hospital from July 2008 to August 2009 were prospectively screened for inclusion in this study. Patients with end-stage kidney disease or kidney transplant, those discharged within 24 hours of enrollment, those transferred from another institution, or those whose urine samples could not be analyzed within four hours of collection were excluded from the study. All adult patients on medical and surgical floors and in intensive care units (ICUs) were screened for AKI on a daily basis using electronic medical records to follow trends in serum creatinine (SCr). Real-time SCr graphs were utilized to detect an increase of 0.3 mg/dL or 50% over 48 hours from a previously determined baseline. For patients who had AKI within 48 hours of admission, baseline SCr was determined from outpatient medical records. For those who developed AKI later in their hospital stay, baseline SCr was defined as the admission SCr. For the few patients whose admission SCr was higher than the SCr at enrollment, then the lowest stable SCr within two days before the AKI episode was used as the baseline. Waiver of written consent permitted the collection of de-identified patient information and urine samples.

Upon enrollment, a 10mL urine sample was obtained either from catheter tubing or from clean catch samples. Samples were centrifuged for 5 minutes at 1500 rpm. The supernatant was decanted, leaving a 0.5 ml residual. The pellet was then resuspended with a pipette. 1 drop of sediment was pipetted onto a glass slide and a coverslip was placed on top. The slide was analyzed under standard light microscopy at low power (10x), high power (40x), and polarization when crystals were identified. Red and white blood cells, RTE cells, RTE casts, granular casts, and hyaline casts were identified

according to standard definitions and their numbers were recorded (40) (see Figure 1). Additionally, the urinary sediment scoring system designed by Perazella et al was used to facilitate subsequent analysis (47). As demonstrated in Table 5, the score was calculated by summing the points that corresponded to the numbers of RTE cells and/or granular casts in the sediment. All investigators who performed the microscopy were instructed by faculty from the Section of Nephrology at Yale through didactic sessions and hands-on demonstrations on the proper identification of important cellular elements in the urine.

Patients were followed until discharge from the hospital, resulting in the collection of demographic data (ie age, gender, and race), as well as information on the patients' relevant comorbidities such as diabetes, hypertension (HTN), peripheral vascular disease (PVD), congestive heart failure (CHF), coronary artery disease (CAD), and chronic kidney disease (CKD). FeNa, urine dipstick analysis and serum creatinine concentrations (baseline, peak, and at time of discharge) were also recorded when available. Primary and secondary outcomes data collected on enrolled patients included in-hospital initiation of dialysis, death during hospitalization, and need for nephrology consultation. Etiologies of AKI were adjudicated retrospectively via chart review. Acute tubular necrosis was defined as AKI that did not respond to fluid resuscitation (ie Scr remained stable or increased) within 48 hours of treatment. Prerenal was defined as AKI that responded to fluid resuscitation with an improvement of Scr within 48 hours of treatment. All other diagnosis were recorded as "other." Results were recorded on a data

Table 5 Urine sediment scoring system based on numbers of granular casts and RTE cells

	Granular casts (per LPF)		
RTE cells (per HPF)	**0 (0 points)**	**1-5 (1 point)**	**≥6 (2 points)**
0 (0 points)	0	1	2
1-5 (1 point)	1	2	3
≥6 (2 points)	2	3	4

Adapted from Perazella et al (47)

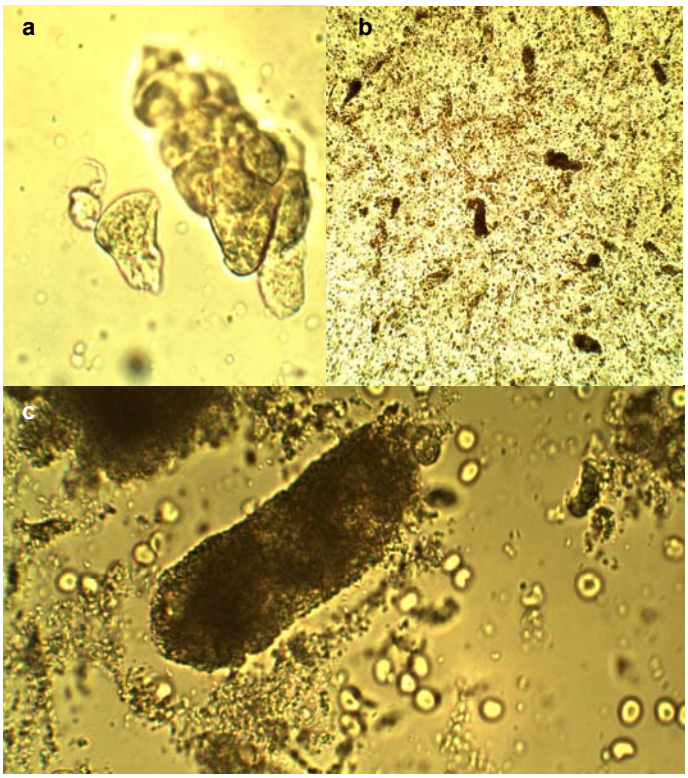


Figure 1 Images of microscopy findings from our study patients
a) RTE cell (high-power view)
b) Granular casts (low-power view)
c) Granular cast (high-power view)

collection form and subsequently entered into TrialDB, an online database.

The primary outcome was the composite outcome of "worsened AKI," defined by an increase in AKIN stage at the time of peak serum creatinine or in-hospital death. Categorical variables were compared via χ^2 analyses and continuous variables were compared using one-way ANOVA. All statistical analyses were performed using SPSS and SAS. The study design and protocol was approved by the Human Investigation Committee at Yale University.

Patricia Peter and Isaac Hall, under the guidance of Chirag Parikh, were involved in establishing study design and methodology. Patricia Peter, Madiha Koraishy, and Isaac Hall contributed to data acquisition and recording of laboratory values and hospital course information, in addition to urine sample collection and microscopy evaluation.

Statistical analysis was performed by Patricia Peter with assistance from Isaac Hall and Umo Iyanam.

RESULTS

Table 6 Baseline characteristics of study population by worsened AKI

Characteristic	N (N %)[A] or mean ± SD (N=165)	Worsened[B] or died (N=49)	Did not worsen or die (N=116)	P[C]
Age (years)	66.1 ±16.2	67.5 ±15.1	65.4 ±16.7	0.461
Gender				0.316
Male	94 (57)	25 (51)	69 (60)	
Female	71 (43)	24 (49)	47 (41)	
Race				0.551
White	126 (76)	37 (76)	89 (77)	
Black	28 (17)	10 (20)	18 (16)	
Other	11 (7)	2 (4)	9 (8)	
Body Mass Index	29.4 ±7.7	30.4 ±8.4	29 ±7.3	0.294
Baseline Scr[D]	1.3 ± 0.5	1.3 ± 0.6	1.3 ± 0.4	0.832
Baseline GFR	65 ± 28	66 ± 31	64 ± 27	0.683
Baseline CKD stage				0.050
Stage 1 (GFR≥ 90)	29 (18)	9 (18)	20 (17)	
Stage 2 (GFR 60-89.9)	51 (31)	19 (39)	32 (28)	
Stage 3 (GFR 30-59.9)	74 (45)	15 (31)	59 (51)	
Stage 4-5 (GFR 0-29.9)	11 (7)	6 (12)	5 (4)	
Past Medical History				
Known CKD	44 (27)	12 (26)	32 (28)	0.799
Hypertension	114 (69)	32 (65)	82(71)	0.445
Diabetes	67 (41)	23 (47)	44 (38)	0.301
CHF	66 (40)	19 (39)	47 (41)	0.835
CAD	68 (41)	17 (35)	51 (44)	0.312
Cirrhosis	14 (9)	5 (10)	9 (8)	0.606
COPD	33 (20)	8 (16)	25 (22)	0.443
Stroke	23 (14)	7 (15)	16 (14)	0.895
Dementia	18 (11)	6 (13)	12 (10)	0.655
Active cancer	37 (22)	18 (38)	19 (16)	**0.003***
OSA	18 (11)	4 (8)	14 (12)	0.486
# of Comorbidities				0.323
None	10 (6)	2 (4)	8 (7)	
One	19 (12)	3 (7)	16 (14)	
Two or more	129 (78)	40 (89)	89 (79)	
Tobacco use				0.214
Never	81 (49)	25 (57)	56 (53)	
Prior	35 (21)	13 (30)	22 (21)	
Current	33 (20)	6 (14)	27 (26)	
Enrollment location				**0.032***
ICU	90 (55)	33 (67)	57 (49)	
Floor	75 (46)	16 (33)	59 (51)	
AKI Stage at enrollment				**0.001***
Stage 1	139 (84)	34 (69)	105 (91)	

Stage 2	26 (16)	15 (31)	11 (10)	

[A] Numbers may not sum to totals due to missing data; Row and column percentages for totals may not sum to 100% due to missing data and rounding. [B] Worsened AKI is defined as progression to a higher AKI stage at time of peak serum creatinine when compared to AKI stage on day of enrollment. [C] P-values obtained from analysis of variance F-test (continuous variable) or $\chi 2$ test (categorical variable). [D] Scr = Serum creatinine. Baseline Scr = Admission Scr. If admission Scr is missing or admission Scr is greater than Scr on the day of enrollment (day 0), then baseline Scr = minimum of Scr collected two days before enrollment (day -2 and day -1).
GFR = Glomerular Filtration Rate, CKD = Chronic Kidney Disease, CHF = Congestive Heart Failure, CAD = Coronary Artery Disease, COPD = Chronic Obstructive Pulmonary Disease, OSA = Obstructive Sleep Apnea, ICU = Intensive Care Unit

165 patients admitted to Yale-New Haven Hospital from July 2008 to August 2009 were prospectively enrolled in this study on the first day of their AKI as determined by standard serum creatinine criteria (see Methods section above). 115 patients were ultimately diagnosed with prerenal AKI, 35 with ATN, and the remaining 15 with other causes of AKI including allergic interstitial nephritis, hepatorenal syndrome, obstructive nephropathy, contrast nephropathy, lupus nephritis, and IgA nephropathy.

49 of these patients experienced the composite outcome of worsened AKI or death. Those who worsened were more likely to have an active cancer diagnosis ($p<0.003$), to be located in the ICU ($p<0.032$), and to have a higher AKI stage at the time of enrollment ($p<0.001$) (see Table 6). Otherwise, no significant differences existed in age, gender, or comorbidities between the two populations. Also of note, there were no differences in baseline kidney function prior to injury between the two populations whether measured as serum creatinine, GFR, or CKD stage.

Patients who worsened were more likely to experience secondary outcomes such as oliguria or anuria during their hospitalization ($p<0.001$), higher peak serum creatinines (3.2 ± 1.5 versus 1.9 ± 0.6, $p<0.001$), higher discharge serum creatinines (2.0 ± 1.4 versus 1.4 ± 0.5, $p = 0.001$), and longer lengths of stay (32.4 ± 33.6 versus 18.2 ± 24.5, $p = 0.003$) (see Table 7). The average AKI stage at peak serum creatinine was 2.4 for those

who worsened and 1.1 for those that did not. Though one would perhaps expect that patients with a higher peak AKI stage would have a greater incidence of worsening or death, no such dose-dependent relationship was observed. Instead there was a relatively even distribution of patients, with 31% of worsening patients peaking at Stage 1, 22% peaking at Stage 2, 22% peaking at Stage 3, and 25% peaking at Stage 3 and requiring dialysis. Also, though differences in AKI duration between the two groups did not achieve statistical significance, patients with worse outcomes had AKI that lasted for 2.7 more days, on average, than those that did not worsen. Patients in the "worsened" group were also more likely to require a nephrology consultation over the course of their hospital stay and to ultimately be diagnosed with ATN.

Table 7 Secondary outcomes in the study population

Secondary Outcome	N (N %) or mean ± SD (N=165)	Worsened[A] or died (N=49)	Did not worsen or die (N=116)	P[B]
Peak Scr[C]	2.3 ± 1.1	3.2 ± 1.5	1.9 ± 0.6	**<.001***
AKI Stage at peak Scr	1.5 ± 0.9	2.4 ± 1.2	1.1 ± 0.3	**<.001***
Stage 1	120 (73)	15 (31)	105 (91)	
Stage 2	22 (13)	11 (22)	11 (10)	
Stage 3	11 (7)	11 (22)	0 (0)	
Stage 3 –Dialysis	12 (7)	12 (25)	0 (0)	
Discharge Scr	1.5 ± 0.7	2.0 ± 1.4	1.4 ± 0.5	**0.001***
Oliguria/Anuria	26 (16)	16 (40)	10 (10)	**<.001***
Nephrology consult	35 (21)	23 (47)	12 (10)	**<.001***
AKI Diagnosis				**<0.001***
Prerenal	115 (21)	20 (41)	95 (82)	
ATN	35 (70)	26 (53)	9 (8)	
Other	15 (9)	3 (6)	12 (10)	
Length of AKI	5.1 ± 8.3	7.3 ± 11	4.6 ± 8	0.249
Length of stay	22.5 ± 28.2	32.4 ±33.6	18.2 ±24.5	**0.003***

[A] Worsened AKI is defined as progression to a higher AKI stage at time of peak serum creatinine when compared to AKI stage on day of enrollment. [B] P-values obtained from analysis of variance F-test (continuous variable) or $\chi 2$ test (categorical variable). [C] Scr = Serum creatinine
ATN = Acute Tubular Necrosis

The microscopy results shown in Table 8 reveal a significant association between worsening AKI and a greater number of granular casts in the urine ($p = 0.027$). Though no association was found between worsening and the number of RTE cells individually, a higher overall microscopy score was found in the sediment of patients who worsened or died ($p = 0.046$). Interestingly, the microscopy score was related to worsening with a dose-dependent trend, as 6% of patients who worsened had a score of 0, 25% had a score of 1, and 49% had a score of 2. This trend did not hold at microscopy scores of 3 or greater, however, which only occurred in 20% of patients who worsened.

Table 8 Microscopy results by worsened AKI

Characteristic	N (N %)[A] or mean ± SD (N=165)	Worsened[B] or died (N=49)	Did not worsen or die (N=116)	P[C]
RTE cells/HPF				0.261
0	35 (21)	7 (14)	28 (24)	
1-5	121 (73)	38 (78)	83 (72)	
WBCs/HPF				0.372
0	42 (26)	9 (18)	33 (28)	
1-5	82 (50)	27 (55)	55 (47)	
6-20	27 (16)	7 (14)	20 (17)	
RBCs/HPF				0.424
0	44 (27)	10 (20)	34 (29)	
1-5	70 (42)	20 (41)	50 (43)	
6-20	27 (16)	11 (22)	16 (14)	
RTE cell casts/LPF				0.234
0	112 (68)	30 (61)	82 (71)	
1-5	53 (32)	19 (39)	34 (29)	
Granular casts/LPF				**0.027***
0	58 (35)	11 (22)	47 (41)	
1-5	95 (58)	32 (65)	63 (54)	
6-20	10 (6)	4 (8)	6 (5)	
WBC casts/LPF				0.580
0	145 (88)	42 (86)	103 (89)	
1-5	20 (12)	7 (14)	13 (11)	
Hyaline casts/LPF				0.942
0	69 (42)	20 (41)	49 (42)	
1-5	60 (36)	17 (35)	43 (37)	
6-20	29 (18)	10 (20)	19 (16)	
Microscopy Score[D]				**0.046***
0	21 (13)	3 (6)	18 (16)	
1	51 (31)	12 (25)	39 (34)	
2	74 (45)	24 (49)	50 (43)	
≥3	19 (12)	10 (20)	9 (8)	

[A] Numbers may not sum to totals due to missing data; Row and column percentages for totals may not sum to 100% due to missing data and rounding. [B] Worsened AKI is defined as progression to a higher AKI stage at time of peak serum creatinine when compared to AKI stage on day of enrollment. [C] P-values obtained from analysis of variance F-test (continuous variable) or $\chi 2$ test (categorical variable). [D] Microscopy score calculated from Table 5.

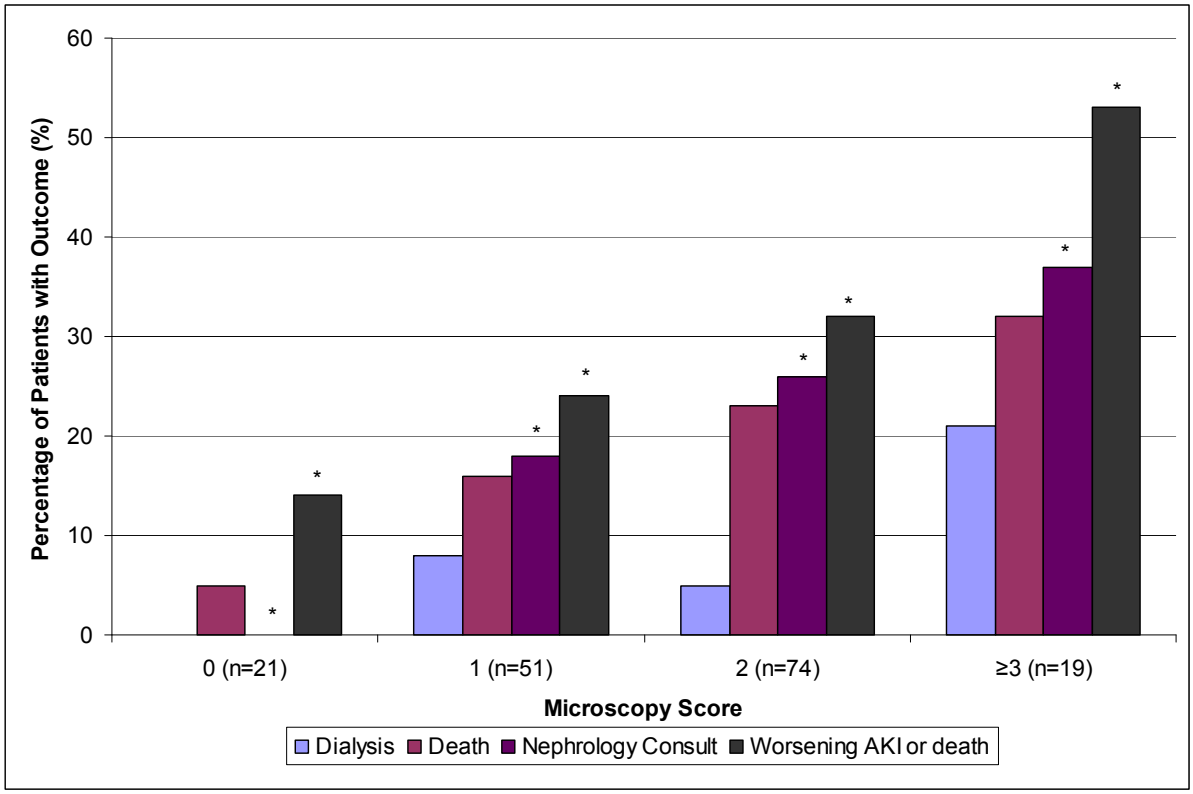
Subgroup analysis was subsequently performed to further investigate the relationship between microscopy scores on day one of AKI and eventual outcomes. Patients with a higher microscopy score on the first day of their AKI were significantly more likely to suffer the composite outcome of worsening AKI or death ($p = 0.046$) and more likely to eventually require a nephrology consultation during the course of their admission ($p = 0.010$), both associations occurring in a dose-dependent manner (see Table 9). The incidence of worsened AKI or death rose consistently from 14% of patients with a microscopy score of 0, 24% with a score of 1, 32% with a score of 2, and finally to 53% in patients with scores of greater than or equal to 3. A similar pattern was found with need for nephrology consultation during hospitalization (with incidences of worsening at 0%, 18%, 26%, and 37% corresponding to scores of 0, 1, 2, and greater than or equal to 3, respectively). Though there was no statistically significant association between microscopy score and the individual outcomes of higher AKI stage at peak serum creatinine, dialysis, death, or ATN diagnosis, there was a noticeable trend of increased incidence of death with successively higher microscopy scores (5%, 16%, 23%, and 32% at scores of 0, 1, 2, and 3 or greater, respectively), a relationship made clear in the graphical representation below (see Figure 2).

Table 9 Subgroup analysis of outcomes by microscopy score

Microscopy[A] Score	Outcome							
	Worsened[B] or died	AKI stage at peak SCr	Dialysis	Death	Nephrology consult	Diagnosis		
						ATN	Prerenal	Other
0 (n=21)	3 (14)	3 (14)	0 (0)	1 (5)	0 (0)	3 (14)	15 (71)	3 (14)
1 (n=51)	12 (24)	9 (18)	4 (8)	8 (16)	9 (18)	8 (16)	40 (78)	3 (6)
2 (n=74)	24 (32)	10 (14)	4 (5)	17 (23)	19 (26)	15 (20)	51 (69)	8 (11)
≥3 (n=19)	10 (53)	8 (42)	4 (21)	6 (32)	7 (37)	9 (47)	9 (47)	1 (5)
P[C] =	**0.046***	0.055	0.078	0.117	**0.010***	0.119		

All values are reported as N (N%). [A] Microscopy score calculated from Table 5. [B] Worsened AKI is defined as progression to a higher AKI stage at time of peak serum creatinine when compared to AKI stage on day of enrollment. [C] P-values obtained from Fisher's exact test using $\chi 2$ analysis.
AKI = acute kidney injury, ATN = acute tubular necrosis, SCr = serum creatinine

Figure 1 Association between increasing microscopy score and outcomes



DISCUSSION

Despite the wealth of research devoted to the field, AKI remains a prevalent disease that has seen little improvement in outcomes over the past several decades. In response, current research has focused on identifying diagnostic tests that will allow for earlier detection of AKI, more accurate determination of etiology, and better prediction of morbidity and mortality from the disease. In this study, we examine the potential value of urine microscopy in achieving at least some of these goals. Our results show that higher numbers of granular casts and RTE cells in the urine sediment of hospitalized patients (as captured in our microscopy score) on the first day of AKI are associated with increased incidence of worsening AKI or death in a dose-dependent fashion. These results build on earlier work from our group in reinforcing the potential utility of urine microscopy in better evaluating and risk stratifying patients with AKI at an early stage of their disease course (40, 41, 47).

Predicting outcomes in AKI has to potential to help guide management as more aggressive interventions could be conducted earlier on patients who are thought to have a poorer prognosis. Additionally, prognostic information would help to better inform physicians about the likelihood that their patients will develop more serious long-term sequela of AKI, helping both patients and their providers to prepare for such consequences. Current efforts to risk stratify patients with AKI rely heavily on established staging criteria since a stepwise increase in risk of death going from Risk to Injury to Failure in the RIFLE criteria has been studied and validated in both small studies and systematic reviews (36, 37, 51). However, the staging criteria are imperfect in predicting outcomes. Firstly, there is a paucity of prospective data supporting the

predictive ability of these staging systems (only about 2% of patients studied were featured in prospective studies) (52). Also, since these criteria were initially designed to simply standardize definitions of AKI, they focus solely on markers of kidney function without consideration for extrinsic factors that might affect outcomes (ie hospital complications, age, etc) (52). Previous attempts at devising AKI-specific severity scores to aid in the determination of prognosis have included variables focused primarily on age, gender, and a variety of hospital course events such as mechanical ventilation, hypotension, sepsis, heart failure, or oliguria with some contributions from biomarkers such as creatinine or urea. Many of these scores have not been externally validated for their ability to accurately predict outcome, and so the search continues for better means of risk-stratifying patients with AKI (53). Our results suggest that as part of the continued effort to identify different stages of AKI severity and to better predict subsequent outcomes, urine microscopy should be considered as a potentially valuable adjunct to clinical and demographic characteristics.

In addition to finding better methods for predicting outcomes, much research in the field of AKI has focused on discovering biomarkers that will more quickly and accurately diagnose AKI early in the course of the disease. The current staging systems rely on serum creatinine as a marker of kidney dysfunction which is believed to be an imperfect and late indicator of true renal injury. This potential delay in diagnosis and treatment of AKI could be contributing in large part to the poor outcomes in this disease (54). Serum creatinine is influenced by many factors extrinsic to renal injury such as age, muscle mass, gender, medications and hydration status. Additionally, serum creatinine requires a steady-state equilibrium to accurately depict kidney function, something that is

often only achieved days after the initial insult. Thus, this need for a more accurate and sensitive marker coupled with an improved understanding of the pathophysiology of kidney injury has led to the identification of many potential urinary biomarkers such as cystatin C, neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), and interleukin-18 (IL-18), several of which have showed much promise improving the accuracy of AKI diagnosis (29, 55-58). As seen in our study, urine microscopy results even early in the course of AKI can provide valuable information about the ultimate degree of renal dysfunction, well before serum creatinine values reach their peak. In this way, urine microscopy when combined with this new class of more powerful biomarkers will greatly enrich our ability to identify and characterize AKI earlier and more precisely.

There are, of course, many limitations to this work. One of the most crucial limitations is the absence of validation of the microscopy findings by an external reviewer and the failure to calculate inter-observer agreement on microscopy findings. Though the microscopy was conducted by investigators who were well-trained to examine urine, they were not subjected to an externally validated test of proficiency. However, the problem of observer bias was minimized in that at the time of microscopy, investigators were not aware that the study would be focused on associating microscopy results with patient outcomes. Other flaws in study design include the absence of a group of patients without AKI to serve as a control and the relatively small sample size that experienced our composite outcome of worsening or death. Additionally, given that creatinine is an admittedly imperfect marker of kidney injury, our microscopy results could in fact be capturing a later time point in the disease course than initially suspected.

Also, though the use of a logistic regression model would perhaps have been a more robust means of statistical analysis, our primary interest was to simply identify potentially significant associations between findings on urine microscopy and outcome. Other work in our lab hopes to go further by then using urine microscopy results along with other potential prognostic indicators such as urine biomarkers to create a powerful new model for understanding and characterizing AKI.

Ultimately, the numbers of granular casts and RTE cells present in the urine of hospitalized patients on the first day of their AKI can be helpful in determining prognosis, including worsening of disease or in-hospital death. A simple diagnostic tool that has long been well-regarded in the field of nephrology for its ability to provide impressive insight into the workings of the kidney, urine microscopy has the potential to expand its role as an invaluable clinical tool in the diagnosis of AKI to include predicting outcomes in AKI, likely as part of a panel of clinical measures and biomarkers. As a component of current and future efforts to find markers that will improve diagnosis and outcome prediction in AKI, urine microscopy will hopefully continue to provide critical information that will help guide management and ultimately improve outcomes of this disease.

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