Original article:

Urine Microscopy-A Golden Tool in Characterizing Acute Kidney Injury

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Abstract:

Aims & Objectives: The purpose of the current study was to evaluate the role of urine microscopy and urine sediment examination in the differential diagnosis and outcome prediction of AKI in hospitalized patients.

Methods: In this cross-sectional study, fresh urine samples were obtained from 100 consecutive patients who were seen for diagnosis of AKI by the nephrology consult service at indoor in Medicine Department at N R S Medical College and Hospital. AKI was defined as a 50% increase in serum creatinine concentration above baseline. In reality, most patients had much more severe increases in serum creatinine concentration at the time of nephrology consultation. The consultant nephrologist was asked to assess the probable cause of AKI at two time points: (1) After clinical assessment of the patient but before urine microscopy (pre-urine microscopy diagnosis) and (2) after patient discharge, renal biopsy, or death (final diagnosis). Instruction included both didactic education about the various cellular elements and casts found in the urine, which included proper collection, preparation, and viewing of the urine.

Results: The urinary sediment scoring system was highly predictive of the final diagnosis of ATN. The odds ratio (OR) for ATN incrementally increased with an increase in severity of the scoring system (all compared with score 0; score 1: OR 9.7, 95% CI 5.3 to 18.6; score ≥2: OR 74.0, 95% CI 16.6 to 329.1. In patients with a high pretest probability of ATN (initial diagnosis of ATN), any granular casts or RTEC (score ≥2) resulted in very high PPV (100%) and low NPV (44%) for a final diagnosis of ATN. In patients with a low pretest probability of ATN (initial diagnosis of prerenal AKI), the lack of granular casts or RTEC on urinary sediment examination had a sensitivity of 0.73 and a specificity of 0.75 for a final diagnosis of ATN. The NPV of lack of granular casts or RTEC in patients with low pretest probability of disease was 91%.

Conclusion: Urine microscopy and examination of the sediment has some advantages on the basis of widespread availability, technique simplicity with conventional equipment, and low cost. Our cross-sectional study of urine microscopy in the setting of hospital-acquired AKI suggests that ATN (sustained AKI) can be confidently differentiated from pre-renal AKI. This was based on determining the presence of granular casts and using a scoring system based on the number of casts and RTEC. Further studies using other urinary indices such as fractional excretion of sodium and biomarkers (*e.g.*, NGAL, IL-18, KIM-1) are warranted to elucidate better the role of granular casts, RTEC, and a scoring system in diagnosis and prognosis of ATN.

Keywords: Urine Microscopy, Urine sediment examination, Acute Kidney Injury.

INTRODUCTION

Acute kidney injury (AKI), defined as an abrupt decline in kidney functions, is a relatively frequent complication and an independent predictor of poor outcomes in hospitalized patients . In fact, AKI occurs in 5–35% of all hospitalized patients and is independently associated with a 2- to 5-fold increased risk of death . In view of the common occurrenceof AKI and its associated poor outcomes, it is critical that potentially reversible AKI is early recognized to

allow for appropriate and timely interventions . As pre-renal AKI (pre-renal azotemia) and acute tubular necrosis (ATN) are the most common causes of AKI in hospitalized patients, and therapies and outcomes for these forms of AKI differ significantly, early clinical differentiation is desirable . The recognitionof AKI is based primarily on clinical history, physical examination, and certain laboratory measurements.Blood urea nitrogen (BUN), serum creatinine,and urine output are traditionally used to diagnose AKI,but they do not provide insight into the cause ofAKI and cannot distinguish between pre-renal AKI and ATN. In the absence of renal biopsy, which is not typically performed in these settings, the differentiation of these two conditions is based on urinary biochemistry and derived indices such as urinary sodium concentration (UNa), fractional excretion of sodium (FeNa), and fractional excretion of urea nitrogen (FeUrea). However, there are limited data concerning the diagnostic strength of these tests in differentiating these two major forms of hospital-acquired AKI . Since the discovery of microscopic elements in the urine in the 19th century, urinalysis has been anessential diagnostic tool in kidney disease . Urine microscopy with urine sediment examination by an experienced nephrologist often provides useful diagnostic information about the histologic state of the kidneys . While most nephrologists use urine microscopy to assess for the presence of glomerular diseases or acute interstitial nephritis, they are lessapt to use this diagnostic test when pre-renal AKI or ATN is clinically suspected. More often, tests such as FeNa and FeUrea are used to differentiate pre-renalAKI from ATN. The purpose of the current study was to critically review the current literature on the role of urine microscopy and urine sediment examination in the differential diagnosis and outcome prediction of AKI in hospitalized patients.

METHOD

In this cross-sectional study, fresh urine samples were obtained from 100 consecutive patients who were seen for diagnosis of AKI by the nephrology consult service at indoor in Medicine Department at N R S Medical College and Hospital. AKI was defined as a 50% increase in serum creatinine concentration above baseline. In reality, most patients had much more severe increases in serum creatinine concentration at the time of nephrology consultation. The consultant nephrologist was asked to assess the probable cause of AKI at two time points: (*1*) After clinical assessment of the patient but before urine microscopy (preurine microscopy diagnosis) and (*2*) after patient discharge, renal biopsy, or death (final diagnosis). Instruction included both didactic education about the various cellular elements and casts found in the urine, which included proper collection, preparation, and viewing of the urine.

At the first time point, the consultants were asked to provide a diagnosis of AKI that fit into one of three categories: ATN, prerenal AKI, or other. The term ATN was used to reflect sustained AKI that did not fit into other categories of intrinsic renal disease. The consultants were instructed to use all available clinical data to make these presumptive diagnoses, including clinical scenario, temporal course of serum creatinine concentration, and response to treatment (including intravenous fluids, discontinuation of or addition of diuretics, vasopressors, and use of steroids). As a general guideline, ATN, although truly a biopsy diagnosis, was defined as a sudden decline in kidney function as manifested by a 50% increase in serum creatinine concentration above baseline that did not respond to fluid resuscitation

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and/or hemodynamic manipulation (*e.g.*, vasopressors) within 48 h of treatment. Prerenal AKI was defined as an abrupt decline in baseline kidney function (as already defined) that improved to ±10% of baseline after fluid resuscitation and/or hemodynamic manipulation within 48 h. The third category was labeled as "other" and included diagnoses such as glomerulonephritis, vasculitis, pyelonephritis, preeclampsia, interstitial nephritis, and obstructive nephropathy.

After the initial consultation, fresh urine was obtained from the patients and were examined within 1 h after voiding. The consultant nephrologists prepared urine sediment samples for analysis. Ten milliliters of urine was centrifuged at 1500 rpm for 5 min in a standard centrifuge (inspected and maintained by Pathology Department at NRS Medical college.Removal by suction of 9.5 milliliters of supernatant urine was performed and followed by gentle manual agitation of the test tubes. A pipette was used to apply a single drop of urine sediment on a glass slide, and coverslip was gently applied. There was no variation in types of glass slides or coverslips used during the study. Samples were examined at low power (×10) and then at high power (×40) on the bright-field microscope. The urine was also viewed with polarization when crystals were identified. Urinary sediment was analyzed for the presence and number of red and white blood cells, RTEC, granular casts, and hyaline casts (all per high-power field). RTEC are defined as size variation (11 to 15 μm in diameter), shape variation (round to columnar), and a well-evident nucleus with nucleoli. Granular casts are defined as fine or coarse granules contained within a cast matrix, whereas hyaline casts are defined as cast matrix without cells and colorless. Granular casts and RTEC per high-power field were recorded on the data collection sheet as present or absent and, when present, were also quantified as the number counted: one to five, six to 10, and >10 and one to five, six to 20, >20, respectively. Other casts, such as hyaline, red blood cell, and white blood cell casts per high-power field, were also recorded. Finally, the diagnosis of AKI was assessed at the second time point (approximately 1 to 4 wk after the initial consultation). The consultants were asked to include all of the available clinical information (especially response to therapy over time and renal biopsy diagnosis) and record the "final diagnosis" of AKI for the individual patients.

For the statistical analyses, we included only patients with ATN and prerenal AKI according to the final diagnosis. We assessed the sensitivity, specificity, positive predictive value (PPV; true positives/true positives + false positives), negative predictive value (NPV; true negative/true negatives + false negatives) of both the initial diagnosis for the final diagnosis and of granular casts for the diagnosis of ATN. We also created a scoring system based on casts and RTEC and evaluated its accuracy for differentiating ATN from prerenal AKI (Table 1). Likelihood ratios (LR) were calculated for a diagnosis of both ATN and prerenal AKI using data from the urine sediment scoring system. The protocol was approved by the ethical committee at West Bengal Health University.

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Table 1. Scoring system based on number of granular casts and RTEC seen per high-power field for differentiating ATN from prerenalAKI[a]

Score	Description
1	RTE cells 0 and granular casts 0
2	RTE cells 0 and granular casts 1 to 5 or RTE cells 1 to 5 and granular casts 0
3	RTE cells 1 to 5 and granular casts 1 to 5 or RTE cells 0 and granular casts 6 to 10 or RTE cells 6 to 20 and granular casts 0

- [a] ATN, acute tubular necrosis; AKI, acute kidney injury; RTEC, renal tubular epithelial cells.

Fresh urine samples were obtained from 130 consecutive patients with AKI, and urinary sediment was examined. In our cohort, 62 (47%) patients had a final diagnosis of ATN, and 53 (40%) patients had prerenal AKI. We excluded 15 (12%) patients with "other" causes of AKI. Using the final diagnosis as the gold standard, the ability of the preurine microscopy diagnosis to distinguish ATN from prerenal AKI was fair (sensitivity 0.76; specificity 0.86; positive LR 5.75). We calculated LR for both ATN and prerenal AKI from the results of microscopic examination (number of granular casts and RTEC) by final clinical diagnosis. These data are shown in Table 2. Using the LR calculated for either ATN or prerenal AKI, the posttest odds of either diagnosis can be estimated after multiplying the pretest odds by the LR from the urine sediment score (posttest odds = pretest odds × LR).

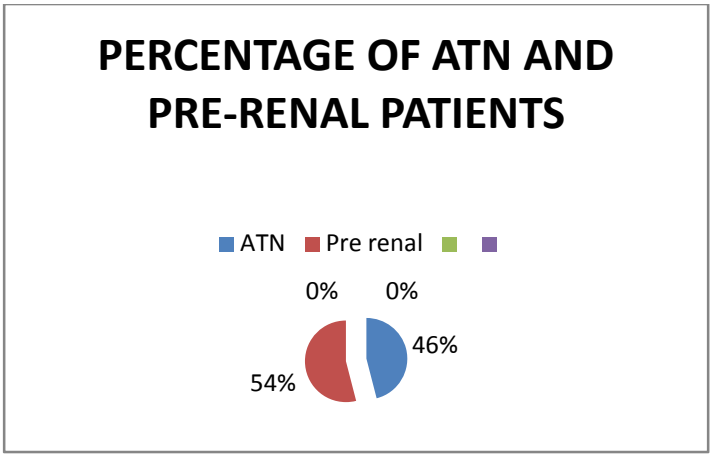

CHART-1:SHOWING THE PERCENTAGE DISTRIBUTION OF ATN AND PRE-RENAL PATIENTS

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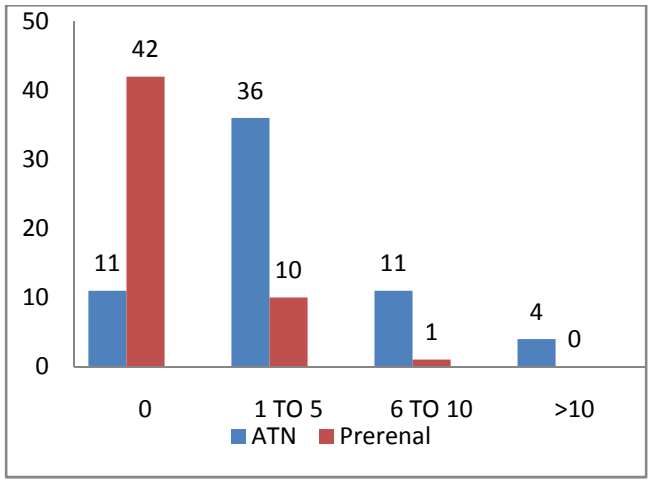

CHART2:-DISTRIBUTION OF PATIENTS BASED ON URINE SEDIMENT SCORE BASED ON GRANULAR CASTS

Table 2. Likelihood ratios for diagnoses of ATN and prerenal AKI based on urine sediment scorea

Urine Findings	**ATN**	**Prerenal AKI**	**LR (ATN)**	**LR (Prerenal AKI)**	PPV	NPV	SENSITIVITY	SPECIFICITY
Granular Casts								
0	11	42	0.25	4.47	79.25	82.26	79.25	82.26
1-5	36	10	2.08	0.35	58.06	81.13	78.26	62.32
6-10	11	1	9.68	0.10				
>10	4	0	∞	0				
TOTAL	62	53						

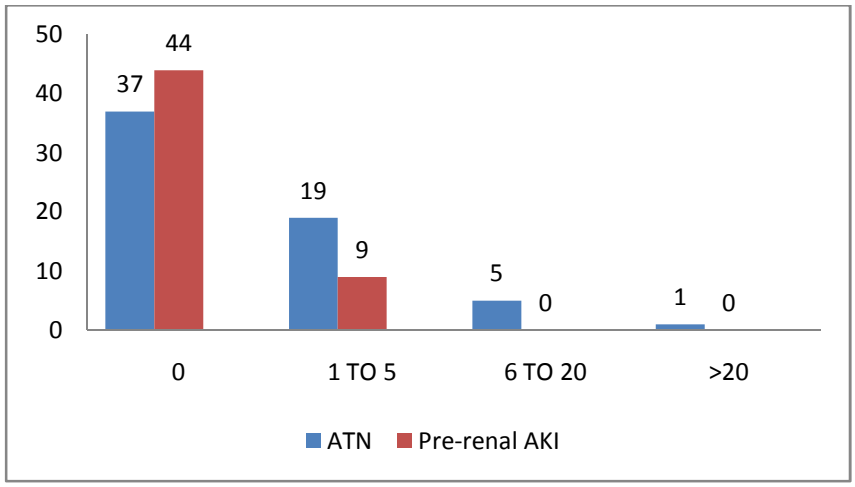

CHART3:DISTRIBUTION OF PATIENTS BASED ON URINE SEDIMENT SCORES BASED ON RTE CELL

Urine Findings (RTEC)	**ATN**	**Prerenal AKI**	**LR (ATN)**	**LR (Prerenal AKI)**
O	37	44	0.72	1.39
1-5	19	9	1.97	0.51
6-20	5	0	∞	0
>20	1	0	∞	0
TOTAL	62	53		

Likelihood ratios for diagnoses of ATN and prerenal AKI based on urine sediment scores

We evaluated the concordance of pre-urine microscopy diagnosis with final diagnosis. The diagnosis was changed in 23% of the patients with prerenal AKI (14patients) to ATN and 14% of the patients with ATN (8 patients) to prerenal AKI (Table 3). Furthermore, we evaluated the role of urine microscopy on the change of diagnosis. We found that granular casts on urine microscopy were present in 85% of the patients whose diagnosis was changed from prerenal AKI to ATN (14 patients), and granular casts were not seen in 67% of the patients whose diagnosis was changed from ATN to prerenal AKI (8 patients; Table 4). In contrast, RTEC were not present in 60% of patients whose diagnosis changed from prerenal AKI to ATN

Table 3 Concordance of pre-urine microscopy diagnosis with final diagnosis

	Prerenal AKI	ATN
Prerenal AKI (60 patients)	46 (77%)	14(23%)
ATN (55 patients)	8(14%)	47 (86%)

The urinary sediment scoring system was highly predictive of the final diagnosis of ATN. The odds ratio (OR) for ATN incrementally increased with an increase in severity of the scoring system (all compared with score 0; score 1: OR 9.7, 95% CI 5.3 to 18.6; score ≥2: OR 74.0, 95% CI 16.6 to 329.1; Tables 5 and and6).6). In patients with a high pretest probability of ATN (initial diagnosis of ATN), any granular casts or RTEC (score ≥2) resulted in very high PPV (100%) and low NPV (44%) for a final diagnosis of ATN. In patients with a low pretest probability of ATN (initial diagnosis of prerenal AKI), the lack of granular casts or RTEC on urinary sediment examination had a sensitivity of 0.73 and a specificity of 0.75 for a final diagnosis of ATN. The NPV of lack of granular casts or RTEC in patients with low pretest probability of disease was 91%.

Table 4:

Parameter	**With Casts (*n* [%])**	Without Casts (*n* [%])
Prerenal AKI → ATN (14patients)	**12(85)**	**2(15)**
ATN → prerenal AKI (8 patients)	**3(33)**	**5(67)**

Table 5.

Association between scoring system and final diagnosis of ATN

Score	**Odds Ratio**	**95% Confidence Interval**
Score 2 *versus* 1	9.7	5.3 to 18.6
Score ≥2 *versus* 1	74	16.6 to 329.1

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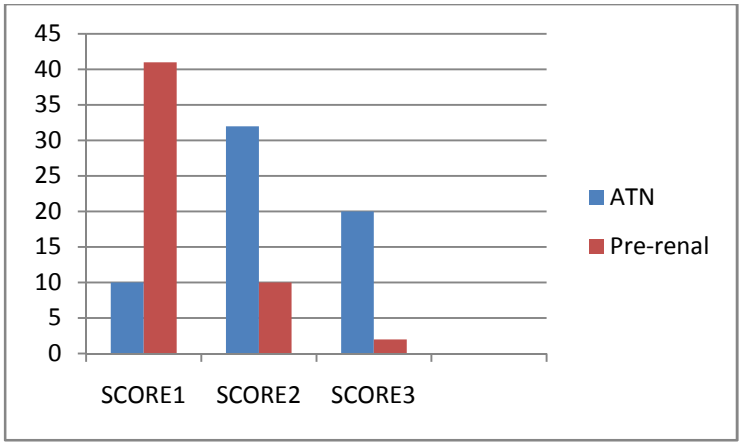

CHART:4-SHOWING THE DISTRIBUTION OF ATN AND PRE-RENAL PATIENTS BASED ON CAST SCORING INDEX

Table 6. Scoring system for ATN *versus* prerenal AKI

Final Diagnosis	**Score (*n* [%])**			**Total No. of Patients**
	1	**2**	**3**	
ATN	10(17%)	32(51%)	20(32%)	62
Prerenal AKI	41(77%)	10(10%)	2(3%)	53

Discussion:

In this study, we evaluated the urine sediment findings and scoring system based on granular casts and RTEC for differentiating ATN from prerenal AKI. Our study demonstrates that urine microscopy on the day of nephrology consultation is indeed a valuable diagnostic tool for strengthening the probability of a diagnosis of ATN. Furthermore, an ATN scoring system is useful for improving the differential diagnosis of ATN *versus* prerenal AKI.

AKI is very common, especially in hospitalized patients,

and it is strongly associated with increased mortality and morbidity (11–13). The most common cause of AKI in hospitalized patients is ATN followed by prerenal AKI (14). Hence, early differential diagnosis of AKI would assist in taking precautions to avoid further renal injury and potentially initiate early treatment to prevent kidney failure. Also, it would avoid worsening of the clinical course with incorrect

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therapies. For example, rapid-volume resuscitation in patients with prerenal AKI as It is generally accepted that urinalysis and urine microscopy with sediment examination are vital for the evaluation of patients with kidney disease, especially in differentiating the causes of AKI. This is particularly true for acute glomerulonephritis, acute interstitial nephritis, and pyelonephritis. Moreover, although urine sediment analysis is considered a part of the clinical workup of kidney disease in hospitalized patients with AKI, its true value in improving diagnosis is not clearly known (1–5). Furthermore, there has been a gradual trend away from using the simple, inexpensive, and rapid modality of urine microscopy in the evaluation of AKI. Unfortunately, it is not known whether this is an acceptable trend (urine microscopy adds nothing to the evaluation of AKI) or a negative trend (loss of useful information for the clinician evaluating the patient with AKI).a result of true volume depletion or judicious intravenous fluid use in patients with ATN would be appropriate management approaches guided by early diagnosis.

The hallmark of the ATN diagnosis is based on clinical history of the patient, physical examination findings, and laboratory analysis (15,16). Previous studies showed that the identification of granular casts and RTEC in the urine sediment analysis correlates well with ATN (17,18). Schentag *et al.* (17) demonstrated that the increase in urinary cast excretion provides information about kidney injury that allows one to adjust aminoglycoside dosages 5 to 9 d before a rise in serum creatinine concentration develops in patients with aminoglycoside nephrotoxicity. Marcussen *et al.* (18) demonstrated that patients with AKI had a high number of granular casts on microscopy compared with those with prerenal AKI. Furthermore, they demonstrated that patients who required dialysis had an increased number of different cast types in the urine sediment. In contrast, a systematic review found that urine microscopy was not beneficial for patients with septic AKI (19); however, many of the studies included in the review had significant limitations.

Currently, no studies have examined the number of granular casts or RTEC that are required to be present to make a diagnosis of ATN. To our knowledge, this is the first study to investigate the role of the number of granular casts, RTEC, and urine scoring system for the diagnosis of ATN. It demonstrates merit in confirming the pre-urine microscopy diagnosis of either prerenal AKI or ATN and changing the diagnosis from one to the other in a significant number of patients. Also, it allows clinicians to use the LR from the appropriate urine sediment score to estimate the posttest probability of their diagnosis on the basis of their pretest probability. For example, after initial assessment of the patient, if the pretest probability of ATN is 50%, or 0.5 (thereby, a pretest probability of prerenal AKI is also 50%), then one can calculate the pretest odds for ATN ($p/1 - P = 0.5/0.5 = 1$) to be 1. With the presence of six to 10 granular casts on urine sediment score (ATN, LR = 9.68), one can calculate a posttest odds for ATN ($1 \times 9.68 = 9.68$). The posttest probability for ATN would be 90.6% (odds/1 + odds = 9.68/1 + 9.68 = 0.906, or 90.6%). The posttest probability of prerenal AKI would be 0.094, or 9.4% (Table 2).

This study has a number of limitations. We did not capture the causes of AKI (*e.g.*, ischemic, septic, nephrotoxic), the clinical characteristics of the patients, or other urinary indices (*e.g.*, fractional excretion of sodium). We also did not obtain biopsies from patients to verify true ATN in patients with AKI sustained for >48 h; therefore, our clinical diagnosis

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of AKI was used as a surrogate as in other studies (20). Although we did provide formal instruction on interpreting the urine sediment, we could not evaluate interobserver variability between the consultant nephrologists for accuracy of correct identification of the urine sediment components. Verification by a second nephrologist may have reduced some of this variability. In addition, the microscopists were not blinded to the initial diagnostic impression or the urine examination; therefore, observer bias may be present in the microscopic examination or the final diagnosis. Our study is also prone to selection bias, because all patients required a nephrology consultation. Finally, because our study focused on hospitalized patients with AKI, we are unable to comment directly on the impact of urine microscopy in the outpatient setting.

Conclusion:

Urine microscopy and examination of the sediment has some advantages on the basis of widespread availability, technique simplicity with conventional equipment, and low cost. Our cross-sectional study of urine microscopy in the setting of hospital-acquired AKI suggests that ATN (sustained AKI) can be confidently differentiated from pre-renal AKI. This was based on determining the presence of granular casts and using a scoring system based on the number of casts and RTEC. Further studies using other urinary indices such as fractional excretion of sodium and biomarkers (*e.g.*, NGAL, IL-18, KIM-1) are warranted to elucidate better the role of granular casts, RTEC, and a scoring system in diagnosis and prognosis of ATN.

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