

ORIGINAL ARTICLE

Saliva urea nitrogen dipsticks to predict acute kidney injury in Malawian trauma patients

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ABSTRACT

Background: Many low-resource settings have limited access to serum creatinine tests necessary for kidney disease identification. Among Malawian patients who are hospitalised after trauma, we evaluated the use of point-of-care saliva urea nitrogen (SUN) dipsticks to predict acute kidney injury (AKI).

Methods: In a nested prospective cohort study, we enrolled hospitalised acute trauma patients aged ≥ 6 months to evaluate AKI (defined by KDIGO criteria) and the test characteristics of SUN to predict AKI.

Results: Among 335 participants (approximately three-quarters able to expectorate and 34% aged ≤ 18 years), 12.5% ($n = 42$) developed AKI. At a SUN threshold of ≥ 40 mg/dL, a positive dipstick test was specific (99.3%) but insensitive (14.3%) in predicting AKI, with a positive predictive value of 75% and negative predictive value of 89%. At this threshold, 2.4% of participants were dipstick-positive (SUN+), and 75% of those had AKI. Reducing the SUN threshold to ≥ 30 mg/dL increased participants who were SUN+ to 5.0% ($n = 16$) but also increased the false positive rate and missed 79% ($n = 33$) of AKI cases. Stratified results showed better performance among adults than children and similar results when comparing participants who could and could not expectorate. There was moderate correlation between categorised BUN values and SUN ($r = 0.53$) but less agreement (weighted kappa 0.27; 95% CI 0.17–0.37).

Conclusions: SUN dipstick testing has good specificity and negative predictive value for ruling out AKI, but poor sensitivity. We found similar results among those who could or could not expectorate a saliva sample.

Keywords: acute kidney injury; saliva urea nitrogen; point-of-care diagnostics; low-resourced settings.

INTRODUCTION

Trauma is a leading cause of mortality for children and young adults (5–29 years of age) [1]. Malawi does not have a national trauma surveillance system but estimates suggest that the country is in the top 20 for fatalities from road traffic accidents and that 14% of all deaths in those 5–24 years of age are due to injuries [2,3]. Acute kidney injury (AKI) is a serious complication of trauma and is

associated with increased mortality [4–8]. In Malawi, large cohort studies have shown that AKI occurred in 12% of admitted paediatric trauma patients and 15% of admitted adult trauma patients, with both populations at relatively high mortality risk [9,10]. However, screening for AKI among trauma patients is quite limited in low-resourced settings, including Malawi.

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COVID-19 has exacerbated healthcare disparities and laid bare many existing inequities, including unequal access to preventative care, vaccines, diagnostics, treatments and staffing. Kidney care worldwide has suffered due to barriers in accessing standard diagnostic tests [11,12]. Many low-resourced settings have limited access to serum creatinine tests necessary for the detection of kidney disease. For AKI, early recognition is essential to allow prompt intervention and the prevention of complications [13].

In places without access to serum creatinine or where there is a delay in obtaining test results (in some places, days to a week), clinicians often must rely solely on urine output to identify AKI. This has raised interest in developing rapid or point-of-care (POC) tests for predicting or screening for AKI at the bedside. Current POC tests for AKI that use creatinine are expensive and require the use of temperature-controlled containers [14,15]. Other tests using novel biomarkers have not yet made it to market [e.g. the neutrophil gelatinase-associated lipocalin (NGAL) dipstick test or require laboratory infrastructure (NephroCheck®)].

An alternative POC test has been developed that measures urea nitrogen levels in saliva, which reflects blood urea nitrogen (BUN) levels [16,17]. These saliva urea nitrogen (SUN) dipsticks are stable at room temperature and are easy to use, being akin to using a standard urine dipstick. SUN dipsticks have not previously been evaluated in patients with trauma or in those with altered mental status. We aimed to evaluate whether a simple POC test, the SUN dipsticks, could be used to predict AKI in Malawian patients hospitalised with trauma.

METHODS

The present study was nested within a larger, prospective cohort investigation to assess the incidence and epidemiology of trauma-related AKI. We evaluated the use of SUN dipsticks (Integrated Biomedical Technology, Elkhart, IN, USA) to predict AKI among hospitalised trauma patients. Data were collected between June and October 2018 at Kamuzu Central Hospital (KCH) in Lilongwe, Malawi, the main tertiary referral hospital for the Central Region. Detailed methods for the larger study have been described previously [18]. The larger study enrolled 337 patients who had suffered acute trauma within 5 days of hospitalisation. The participants selected had an expected hospitalisation duration >24 hours, were aged ≥6 months and had a weight >3 kg. An additional inclusion criterion for the present study was the provision of a saliva sample (whether expectorated or not) during the initial three days of hospitalisation. Participants were excluded if they (or caregivers when required for consent) did not speak English or Chichewa (the Malawian official and national languages, respectively) or if patients had a history of kidney disease.

The primary outcome of interest was the validity of the SUN dipsticks to predict AKI, defined by creatinine-only Kidney Disease Improving Global Outcomes (KDIGO) criteria [13], within the first three days of hospitalisation. Urine output is unreliable and rarely documented in our setting. The creatinine-only KDIGO criteria define AKI by a rise in serum creatinine ≥0.3 mg/dL within 48 hours or ≥50% increase from baseline [13]. Baseline creatinine measurements were not available for any of the participants, and therefore baseline values were estimated using the Modification in Diet of Renal Disease (MDRD) equation for standardised creatinine and back calculating the creatinine assuming an estimated glomerular filtration rate (eGFR) of 75 mL/min/1.73 m² [13]. For paediatric patients, the Schwartz equation was used and assumed a baseline eGFR of 120 mL/min/1.73 m² as this has been shown more accurately to predict baseline kidney function in children [18,19]. Serum and saliva samples were collected on admission and again on hospital day three, if the patient remained alive and hospitalised.

Trained data clerks collected unstimulated saliva from participants without altered mental status and in those old enough to cooperate. Participants refrained from eating or drinking 15 minutes prior to saliva collection. At least 0.5 mL of saliva was collected in disposable paper cups, and the SUN dipstick was submerged in the liquid portion to moisten the colour pad. When participants had altered mental status or were unable to expectorate the saliva, the dipstick was held on the buccal mucosa for 2–3 seconds. The dipstick test pad contains a urease enzyme which cleaves urea when moistened with saliva, leading to the formation of ammonia and hydroxyl ions, resulting in a change in pH. This is detected by a pH indicator which consequently changes the colour of the test pad. After one minute, the colour of the test pad is compared with six reference pads to indicate the SUN concentration as follows: 5 mg/dL (test pad #1), 10 mg/dL (test pad #2), 20 mg/dL (test pad #3), 30 mg/dL (test pad #4), 40 mg/dL (test pad #5) and ≥50 mg/dL (test pad #6).

All serum creatinine and BUN testing occurred in the main research laboratory of the University of North Carolina Project Malawi, located on-site at KCH. The laboratory is state-of-the-art with a constant supply of reagents, daily quality control and assurance testing, and backup generators. Testing occurred on a Roche cobas C311 analyser (Basel, Switzerland) and used a Jaffe method for creatinine measurement, with the assay calibrated to isotope dilution mass spectrometry (IDMS)-traceable reference materials. BUN was measured using the urease method. For assessing the correlation and agreement with SUN, BUN was converted from a continuous variable into six categories (0–7, 8–15,

16–25, 26–35, 36–45, ≥46, all in mg/dL) to correspond to the six categories of SUN.

Additional data available for analysis included sociodemographic variables, comorbidities (self-reported or based on laboratory testing), and injury-related data. The status of human immunodeficiency virus infection was missing for 73% of the participants and is not reported. Hospital-associated outcomes were also recorded as either discharged alive, died in hospital, or left against medical advice. The length of hospital stay was also recorded.

The sample size was determined for the larger cohort study and this nested analysis used all available participants who met inclusion and exclusion criteria. Descriptive statistics were used to summarise the sociodemographic and injury-related characteristics, and comorbidities of the cohort. Chi-squared, Fisher's exact and Wilcoxon rank-sum tests were used to compare groups with and without AKI. To determine the test characteristics of SUN to predict AKI, we calculated sensitivity, specificity, and positive and negative predictive values at a variety of thresholds for SUN. We also compared subgroups (adults versus children, and those able or unable to provide expectorated saliva samples). A sensitivity analysis also assessed the ability of BUN to predict AKI in a similar manner. To assess any relationship between BUN and SUN, Pearson's correlation coefficient was calculated, and agreement was determined

by weighted kappa statistics using the categories of BUN as described above. Data were double-entered into REDCap [20] and all statistical analyses were performed using SAS version 9.4 (SAS Institute, Inc., Cary, NC).

The institutional and ethical review boards of UNC and Malawi's National Health Science Research Committee approved the study. All participants, or their caregivers, provided informed consent.

RESULTS

Among 335 eligible participants, 13% (n = 42) developed AKI (Table 1). Approximately three-quarters were able to expectorate, and the remainder had saliva obtained passively. The majority (78%) were male, and 34% were aged ≤18 years. The majority (80%) presented within 24 hours of trauma, and common traumas included road traffic accidents (43%), assaults (19%), and burns (13%). There was a high prevalence of anaemia (66%) and a low prevalence of self-reported comorbidities (e.g. hypertension in 1.7% and diabetes in 0.3%). Twenty-six patients (8%) died during hospitalisation.

No adverse events were reported in obtaining the saliva samples. SUN correlated reasonably well with the BUN measurements (Figure 1). For clarity, one outlier was omitted from the figure (but not from the data analyses) – on hospital day three, this patient had a SUN dipstick mea-

Table 1. Demographics and outcomes of hospitalised Malawian trauma patients.

	Total 335	AKI 42 (12.5)**	No AKI 293 (87.5)	P value	Missing data (n)
Male	262 (78)	34 (81)	228 (78)	0.67	1
Age (years), median (IQR)	25 (11–38)	31 (19–43)	24 (11–37)	0.08	2
Children ≤18 years	113 (34)	10 (24)	103 (35)	0.14	2
Injury within 24 hours of hospitalisation	267 (80)	39 (93)	228 (78)	0.04	4
Type of trauma					
Burn	43 (13)	9 (21)	34 (12)	0.09	7
Motor vehicle accident	143 (43)	18 (43)	125 (43)	0.92	7
Assault	62 (19)	8 (19)	54 (18)	1.00	7
Truncal injury	99 (30)	17 (41)	82 (28)	0.08	2
Multiple injuries	183 (55)	29 (69)	154 (53)	0.03	2
Comorbidities					
Anaemia	220 (66)	37 (88)	183 (63)	<0.001	0
Malaria	28 (8.4)	1 (2.4)	27 (9.2)	0.23	5
Sickle cell trait	25 (7.5)	5 (12)	20 (6.8)	0.22	14
Saliva sample collection method					
Expectorate, on admission	260 (78)	30 (71)	230 (79)	0.30	0
Expectorated, day 3	258 (77)	35 (83)	223 (76)	0.20	71
Length of hospitalisation, median (IQR)*	11 (6–27)	14 (8–46)	10 (6–26)	0.06	22
In-hospital mortality	26 (7.8)	9 (21.4)	17 (5.8)	<0.001	21

*Length of hospitalisation presented among survivors only, n = 287. **Percentage in brackets. Abbreviations: AKI, acute kidney injury; SUN, saliva urea nitrogen.

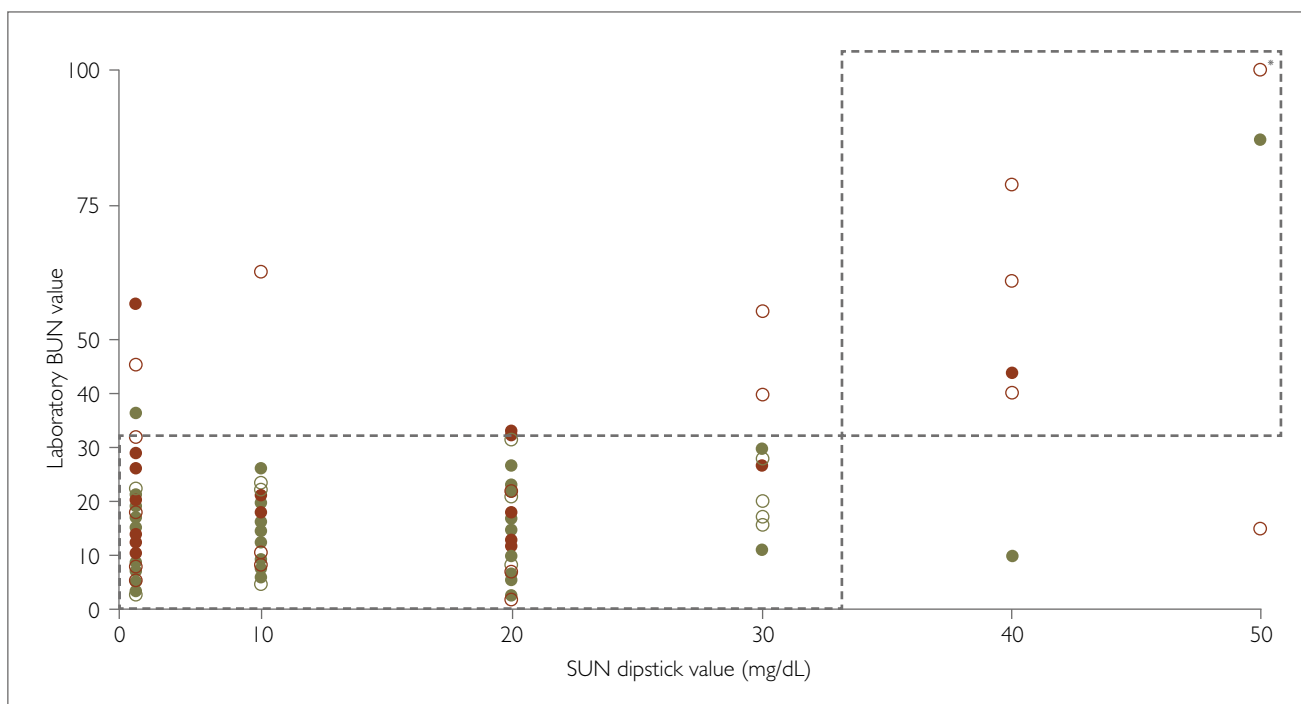


Figure 1. Saliva urea nitrogen dipsticks compared to blood urea nitrogen values by AKI status. Samples collected on admission are represented by filled circles, and those collected on day 3 by open circles. Maroon circles represent patients who developed acute kidney injury, whereas green circles represent patients who did not.

*One outlier was removed from the figure, see text.

Abbreviations: BUN, blood urea nitrogen (laboratory measurement); SUN, saliva urea nitrogen (performed with a dipstick at the bedside).

Table 2. Testing characteristics of saliva urea nitrogen dipsticks for predicting acute kidney injury in hospitalised Malawian trauma patients during the first three days of admission.

SUN values	Admission SUN*					Highest SUN**				
	AKI	Sensitivity	Specificity	PPV	NPV	AKI	Sensitivity	Specificity	PPV	NPV
5	22 (of 234)	52.4	26.9	9.4	79.6	13 (of 190)	31.0	39.6	6.8	80.0
10	13 (of 68)	31.0	81.0	19.1	89.0	13 (of 93)	31.0	72.7	14.0	88.0
20	5 (of 24)	11.9	93.5	20.8	88.0	7 (of 36)	16.7	90.1	19.4	88.3
30	1 (of 3)	2.4	99.3	33.3	87.5	3 (of 8)	7.1	98.3	37.5	88.1
40	1 (of 2)	2.4	99.7	50.0	87.6	4 (of 5)	9.5	99.7	80.0	88.5
50	0 (of 1)	0.0	99.7	0.0	87.3	2 (of 3)	4.8	99.7	66.7	88.0

*Missing n = 3 participants. **Highest value within first three days of hospitalisation.

Abbreviations: AKI, acute kidney injury; NPV, negative predictive value; PPV, positive predictive value; SUN, saliva urea nitrogen.

surement of 50 mg/dL and a laboratory BUN of 207 mg/dL. In our study population, only 10 patients had BUN \geq 40 mg/dL (3% of the total). Of the 42 patients with AKI, 9 (21%) had BUN \geq 40 mg/dL. Only 8 patients had SUN dipsticks of 40 mg/dL or 50 mg/dL, yet 75% of those (6 patients) had concurrent BUN elevations (\geq 40 mg/dL). The correlation coefficients for SUN and BUN were 0.37 on hospital day 1, and 0.53 on hospital day 3. The agreement between BUN and SUN was less congruent, with a day 1 kappa statistic of 0.18 (95% CI 0.10–0.26) and a day 3 kappa statistic of 0.27 (95% CI 0.17–0.37).

The SUN dipsticks, at a threshold of \geq 40 mg/dL, were specific (99%) but insensitive (14%) in predicting AKI, with a positive predictive value of 75% and negative predictive value of 89% (Table 2). At this threshold, 2.4% were SUN+, and 75% of those had AKI. Reducing the SUN threshold to \geq 30 mg/dL increased the SUN+ proportion to 5% (n = 16) but also increased the false positive rate and missed 79% (n = 33) of AKI cases. Sensitivity analysis comparing those who expectorated and those unable to do so (samples collected passively) did not reveal significant differences. However, there was a better test performance

Table 3. Testing characteristics of saliva urea nitrogen dipsticks for predicting acute kidney injury in hospitalised Malawian trauma patients, stratified by age.

Positive SUN dipstick test*	AKI	Sensitivity	Specificity	PPV	NPV
Total cohort	6 (of 8)	14.3	99.3	75.0	89.0
Children <18 years	0 (of 1)	0.0	99.0	0.0	92.5
Adults ≥18 years	6 (of 7)	17.7	99.5	85.7	87.2

*A positive SUN result is defined as a value of 40 mg/dL or 50 mg/dL (n = 8). Only 6 of the 42 episodes of AKI had a SUN value of 40mg/dL or 50mg/dL. Abbreviations: AKI, acute kidney injury; NPV, negative predictive value; PPV, positive predictive value; SUN, saliva urea nitrogen.

among adults compared to children (Table 3). Similar test characteristics were seen when using BUN to predict AKI, with a sensitivity of 9.5% and a specificity of 99.7% when using a threshold of ≥ 36 mg/dL.

DISCUSSION

In this Malawian trauma cohort, there was a high incidence of AKI and SUN dipsticks proved to be an easy tool to administer at the bedside. However, even though the specificity and negative predictive value were high (99.3% and 89.0%, respectively), the sensitivity was poor at 14.3%. This is similar to the findings from a cohort of obstetric patients at high risk of AKI, with reasonable specificity (97.3%) but poor sensitivity (12.8%) [21].

Previous studies on SUN dipsticks in adults without altered consciousness reported better test characteristics than our cohort [22,23]. Similar to our study, a paediatric investigation in Sudan found that the SUN dipstick measurements correlated well with BUN, and also that the SUN dipsticks test was a poor predictor of AKI [16]. SUN dipsticks have not been well tested in populations unable to expectorate saliva samples. In our study, whether a sample was expectorated or not did not alter the results, suggesting that dipsticks may be of value in testing patients with altered mental status.

Urea nitrogen concentrations – whether in blood or saliva – may not be a good marker for AKI as they are affected by hydration status and protein intake, making their applicability in cohorts with varying degrees of dehydration and malnutrition problematic [24]. A study from China suggested only a modest discriminatory value of BUN to predict AKI in post-liver transplant recipients, with a sensitivity of 54% and specificity of 78% [25]. Similar to serum creatinine, increases in BUN (outside the normal range) can lag 24–72 hours after a major loss of GFR, so SUN may be expected to have this inherent limitation as well. Hence, there is a growing plethora of literature in higher-resourced settings for earlier biomarkers to predict AKI prior to rises in serum creatinine.

Our typical paradigm of a laboratory-based diagnosis of AKI may need to shift to a more practical one in settings

that continue to lack access to advanced laboratory infrastructure [11,26]. During large earthquakes, retrospective analyses have assessed the utility of the standard urine dipstick test for haematuria in predicting subsequent AKI and assisting with fluid management, particularly in cases of rhabdomyolysis. These studies vary in their definition of AKI and are limited by retrospective designs with missing data, but overall suggest that, for rhabdomyolysis-induced AKI, standard urine dipsticks could be a useful tool [27,28]. POC tests for serum creatinine are appealing as they use markers that clinicians are familiar with and essentially adapt existing diagnostics to the bedside. However, these POC machines are expensive and require strict temperature-controlled settings for containers and cartridges, which may limit their use in limited-resource settings [14,15,29]. The SUN dipstick has several advantages in that it is stable at room temperature, easy to use and relies on a known marker (urea nitrogen), which clinicians can interpret easily. Dipsticks that use emerging biomarkers, such as NGAL, are only recently being evaluated [30,31].

Our study has some limitations. First, given the cost and lack of routine testing for serum creatinine, we could diagnose AKI based on only two serially collected serum creatinine values during the first three days of hospital admission. Second, as with many AKI studies, our patients had no baseline serum creatinine measurements and we therefore estimated baseline creatinine by back calculation from presumed baseline eGFR, as recommended by international guidelines for adults and standard reference values for children; this approach has been validated previously in various African populations.

CONCLUSIONS

The use of SUN dipsticks for predicting AKI in trauma cohorts requires further study before any clinical application. Our study suggests good specificity and negative predictive value for ruling out AKI, but poor sensitivity. We found similar results among those who could or could not expectorate a saliva sample. SUN dipsticks may still hold clinical utility in other populations or for purposes such as monitoring the trend of kidney function in individual patients, for example, in response to therapies.

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Conflict of interest

The authors have no conflict of interest to declare.

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