Furosemide Stress Test and Biomarkers for the Prediction of AKI Severity

Jay L. Koyner,* Danielle L. Davison,† Ermira Brasha-Mitchell,† Divya M. Chalikonda,† John M. Arthur,‡ Andrew D. Shaw,§ James A. Tumlin,‖ Sharon A. Trevino,* Michael R. Bennett,¶ Paul L. Kimmel,** Michael G. Seneff,† and Lakhmir S. Chawla††

*Section of Nephrology, Department of Medicine, University of Chicago, Chicago, Illinois; †Department of Anesthesiology and Critical Care Medicine and **Department of Medicine, George Washington University Medical Center, Washington DC; ‡Division of Nephrology, Department of Medicine, Medical University of South Carolina, Charleston, South Carolina; §Department of Anesthesiology, Vanderbilt University Medical Center, Nashville, Tennessee; ‖Renal Division, University of Tennessee College of Medicine at Chattanooga, Chattanooga, Tennessee; ¶Division of Nephrology and Hypertension, Cincinnati Children's Hospital, Cincinnati, Ohio; and ††Department of Medicine, Division of Intensive Care Medicine and Division of Nephrology, Washington DC Veterans Affairs Medical Center, Washington, DC

ABSTRACT

Clinicians have access to limited tools that predict which patients with early AKI will progress to more severe stages. In early AKI, urine output after a furosemide stress test (FST), which involves intravenous administration of furosemide (1.0 or 1.5 mg/kg), can predict the development of stage 3 AKI. We measured several AKI biomarkers in our previously published cohort of 77 patients with early AKI who received an FST and evaluated the ability of FST urine output and biomarkers to predict the development of stage 3 AKI (n=25 [32.5%]), receipt of RRT (n=11 [14.2%]), or inpatient mortality (n=16 [20.7%]). With an area under the curve (AUC)±SEM of 0.87±0.09 ($P<0.0001$), 2-hour urine output after FST was significantly better than each urinary biomarker tested in predicting progression to stage 3 ($P<0.05$). FST urine output was the only biomarker to significantly predict RRT (0.86±0.08; P=0.001). Regardless of the end point, combining FST urine output with individual biomarkers using logistic regression did not significantly improve risk stratification (ΔAUC, $P>0.10$ for all). When FST urine output was assessed in patients with increased biomarker levels, the AUC for progression to stage 3 improved to 0.90±0.06 and the AUC for receipt of RRT improved to 0.91±0.08. Overall, in the setting of early AKI, FST urine output outperformed biochemical biomarkers for prediction of progressive AKI, need for RRT, and inpatient mortality. Using a FST in patients with increased biomarker levels improves risk stratification, although further research is needed.

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AKI is the most common reason for inpatient nephrology consultation and carries an increased risk for morbidity and mortality.[1–3] Despite a dramatic increase in the incidence of AKI over the last decade, physicians still lack the clinical tools to determine the likelihood of AKI progression (defined as a worsening of AKI stage, such as progressing from stage 1 to stage 2 or 3) for those with early AKI.[4] Improving patient risk stratification will be crucial as therapeutic trials aim to enroll patients with early AKI who are at highest risk for RRT or inpatient death.[5] In addition, the past decade has seen an explosion of studies seeking to discover new biomarkers of AKI.[6,7] In patients with early AKI (*e.g.*, stage 1), several biomarkers of AKI, including plasma neutrophil gelatinase-associated lipocalin (NGAL), urinary IL-18, tissue inhibitor of

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Correspondence: Dr. Lakhmir S. Chawla, 50 Irving Street NW, Washington, DC 20422. Email minkchawla@gmail.com.

metalloproteinases (TIMP-2) and IGF-binding protein-7 (IGFBP-7) have demonstrated variable ability to predict AKI progression.[8–12] However, despite intense investigation, the utility of these and other biomarkers remains unclear, and most nephrologists and intensivists do not have clinical access to these assays.

We recently demonstrated that the 2-hour urine output after a standardized high-dose furosemide stress test (FST, 1 mg/kg of furosemide in naive patients or 1.5 mg/kg in those with prior exposure) in clinically euvolemic patients with early AKI has the predictive capacity to identify those with severe and progressive AKI.[13] The area under the receiver-operating characteristic curve (AUC) for the urine output 2 hours after FST to predict progression to AKI Network (AKIN) Stage-3 AKI in 77 patients (±SEM) was 0.87±0.09; P=0.001. The ideal cutoff for predicting progressive AKI during these first 2 hours was a urine volume <200 ml (100 ml/hr) with a sensitivity of 87.1% and a specificity of 84.1%.[13]

In the current study we compare the performance of several biomarkers of AKI, including the fractional excretion of sodium (FeNa), urine and plasma NGAL, urine albumin-to-creatinine ratio, urinary IL-18, kidney injury molecule-1 (KIM-1), TIMP2, IGFBP-7, and uromodulin, with that of FST for the prediction of several clinical end points, including progressive AKI, need for RRT, and inpatient mortality.

RESULTS

As previously reported, 25 of 72 (32.5%) patients progressed to Acute Kidney Injury Network (AKIN) stage 3 AKI after evaluation with FST.[13] Table 1 lists the clinical characteristics of those with and without progressive AKI. Patients with progressive AKI were more likely to have cirrhosis, had lower pre-furosemide urinary flow rates, had evidence of more severe tubular injury before FST (according to the George Washington University–Urinalysis Scoring System [GWU-USS]), and were more likely to have AKIN stage 2 at the time of FST.

Prediction of Progression to AKIN Stage 3

The 2-hour urine output (UOP) after FST was associated with the development of AKIN stage 3 AKI with an AUC (±SEM) of 0.87±0.05 (P<0.001) (Table 2). Several biomarkers were also able to significantly predict progression to stage 3, but no individual biomarker provided an AUC>0.80. Plasma NGAL performed the best, with an AUC of 0.75±0.08 (P=0.007). When compared head to head, the AUC of FST was significantly higher than that of all other biomarkers with the exception of plasma NGAL (Table 2). Combining individual biomarkers with the 2-hour UOP demonstrated that some biomarkers improved the risk prediction of AKIN stage 3, while other biomarkers diminished the FST's ability to predict AKI progression (Table 2). However, despite increases in the AUC, including an AUC with IGFBP7×TIMP2+2-hour UOP of 0.90±0.06 (P=0.20), the FST alone and the biomarkers combined with FST did not significantly differ.

Prediction of Receipt of RRT

Eleven patients (14.2% of the total cohort) required RRT following FST (Table 1). Table 3 demonstrates the AUCs for the FST and biomarkers to predict the need for RRT. Two-hour urine output outperformed all individual biomarkers, providing an AUC of 0.86±0.08 (P<0.001), with no single biomarker providing a statistically significant P value or an AUC>0.65. FST significantly outperformed several of the individual biomarkers, including urine NGAL, IL-18, KIM-1, and IGFBP-7 for predicting the receipt of RRT. Combining an individual biochemical biomarker with the FST did not significantly increase the AUC for the prediction of RRT above that seen with FST alone; several biomarkers combinations decreased the AUC compared with the AUC for FST alone (Table 3).

Prediction of Inpatient Death

Sixteen (20.7%) of the entire cohort died in the hospital. Those with AKIN stage 3 (n=9 [36%]) were more likely to die than nonprogressors (n=7 [13.4%]; P=0.04) (Table 1). Two-hour UOP after FST provided an AUC of 0.70±0.09 (P=0.02) for inpatient death; FST and KIM-1 were the only biomarkers displaying significant relationships with this end point. Despite a higher AUC, FST was only significantly better than FeNa at forecasting inpatient death (Table 4). Combining biomarkers with FST provided no significant improvement over FST in mortality prediction.

Prediction of Composite Endpoint of Death or AKIN Stage 3

Thirty-two patients (41.5% of the total cohort) met the composite end point of death or progression to AKIN stage 3. The 2-hour UOP significantly predicted this composite end point, with an AUC of 0.81±0.06 (P<0.001) (Table 5). Plasma and urine NGAL, urine KIM-1, and IGFBP7×TIMP-2 also significantly predicted death or progression (AUC range, 0.64–0.69). FST provided a significant increase in AUC for the prediction of this end point compared with IL-18, KIM-1, uromodulin, and urine creatinine. While combining uromodulin and FST increased the AUC to 0.85±0.06, there was no significant difference between FST and biomarker combinations (Table 5).

Prediction of Outcomes Using Biomarker Cutoffs

Forty-four (57.1%) patients had a urine NGAL concentration >150 ng/ml before FST. In this group, FST significantly predicted the development of all four outcomes (progression to stage 3, receipt of RRT, inpatient death, and the composite end point). The AUC for the prediction of RRT was 0.91±0.06 (P<0.001), while the AUC for the composite end point of stage 3 AKI or death was 0.89±0.06 (P<0.001) (Table 6). There were 33 patients (41.5%) with a pre-FST TIMP-2×IGFBP-7 concentration >0.3. In this subset of patients, FST provided an increased AUC for the prediction of progression to stage 3 AKI (0.90±0.06; P<0.001) and receipt of RRT (0.91±0.08; P<0.001) (Table 6).

Table 1. Patient characteristics

Variable	Entire Cohort (*n*=77)	Nonprogressors (*n*=52)	AKIN Stage 3 Progressors (*n*=25)	*P* Value
Demographics				
Age (yr)	65.3±1.6	63.8±2.2	68.2±1.9	0.13
Median weight (IQR) (kg)	82.0 (68.5–96.8)	80.0 (68.9–94.9)	85.0 (68.3–99.8)	0.64
Men (%)	42.8	36.5	56	0.14
Race, *n* (%)				0.20
African American	44 (57.1)	29 (55.6)	15.0 (60)	
White	23 (29.9)	15 (28.8)	8 (32)	
Hispanic	10 (13.0)	8 (15.4)	2 (8)	
Comorbidities, *n* (%)				
CKD	24 (31)	17 (32.7)	7 (28.0)	0.80
Hypertension	60 (78)	41 (78.8)	19 (76)	0.78
Cirrhosis	4 (5)	0 (0)	4 (16)	0.01
Congestive heart failure	25 (32.5)	15 (29)	10 (40)	0.44
Diabetes mellitus	35 (44)	22 (41.5)	13 (52)	0.47
Nephrotoxic exposures, *n* (%)				
NSAIDs	8 (10)	6 (2)	2 (1)	0.99
Aminoglycosides	1 (1)	0 (0)	1 (4.0)	0.63
Amphotericin	2 (3)	2 (4)	0 (0)	0.99
Contrast medium	21 (27)	15 (28.8)	6 (23.1)	0.79
Cardiac surgery	9 (11.7)	6 (11.5)	3 (12.0)	0.99
Sepsis	15 (19.5)	12 (23.1)	3 (12.0)	0.36
Clinical data				
Baseline eGFR (ml/min per 1.73 m^2)	68.6±4.1	60.0±8.8	73.3±4.2	0.15
Baseline urine flow rate (ml/hr)	74.6±11.6	95.7±16.3	29.7±4.2	0.001
Furosemide naive, *n* (%)	29 (37.7)	23 (44.2)	6 (24)	0.13
Median furosemide dose (IQR) (mg)	100 (80–131.5)	100 (73.3–132.3)	100 (87.5–135)	0.35
Urine cast score	2.3±0.13	2.1±0.16	2.7±0.23	0.05
Median FeNa (IQR) (%)	0.58 (0.14–1.1)	0.60 (0.13–1.4)	0.51 (0.13–1.0)	0.96
CV SOFA score	1.16±0.03	1.05±0.2	1.5±0.4	0.37
APACHE II score	17.8±1.11	16.5±1.2	21.6±2.5	0.08
AKIN stage at enrollment, *n* (%)				
Stage 1 (total)	41 (53.2)	34 (65.4)	7 (28.0)	
Urine output criteria	15 (19.4)	11 (21.1)	4 (16.0)	
Serum creatinine criteria	38 (49.4)	32 (61.5)	6 (24.0)	
Stage 2 (total)	36 (46.7)	18 (34.6)	18 (72.0)	0.003
Urine output criteria	26 (33.8)	11 (21.2)	15 (60.0)	
Serum creatinine criteria	15 (28.8)	10 (19.2)	5 (20.0)	
Outcomes				
Death	16 (20.7)	7 (13.4)	9 (36.0)	0.04
AKIN stage 3	25 (32.4)	NA	25 (100)	NA
RRT	11 (14.2)	NA	11 (44.0)	NA
Death/AKIN	32 (41.5)	7 (13.4)	25 (100)	0.001

Data presented as mean±SEM unless otherwise noted. NSAID, nonsteroidal anti-inflammatory drug; CV SOFA, Cardiovascular Sequential Organ Failure Assessment; APACHE, Acute Physiology and Chronic Health Evaluation; NA, not applicable.

DISCUSSION

We compared the ability of urine output after a standardized furosemide challenge to several contemporary AKI biomarkers, measured just before furosemide administration, to predict several clinical outcomes in 77 patients with early AKI. Our data demonstrate that urine output in the first 2 hours after FST outperforms several biomarkers of AKI for the prediction of AKI progression and future receipt of RRT. Specifically, FST was significantly better than our complete panel of urinary biomarkers at predicting progression to AKIN stage 3. The addition of biomarkers to FST results did not provide any additional benefit. Similarly, FST outperformed all other biomarkers in predicting the end point of receipt of RRT and inpatient death.

Interestingly, when we used prespecified biomarker cutoffs and assessed the performance of FST to predict patient outcomes in a "high-risk" subset, we saw increases in the AUC compared with FST alone. These analyses were modeled on clinical care of patients with myocardial infarction and pulmonary embolus in which a noninvasive tissue injury biomarker, such as serum troponin or D-dimer, is measured to assist in risk stratification and assess the need for more invasive testing (*e.g.,* cardiac stress

Table 2. AUCs for prediction of progression to AKI stage 3

Biomarker	AUC±SEM	*P* Value for Biomarker Alone	*P* Value Compared With FST alone	AUC of Biomarker and FST±SEM	*P* Value for Biomarker and FST Compared With FST Alone
FST (2-hr UOP)	0.87±0.05	<0.001	NA	NA	NA
Urine NGAL	0.65±0.06	0.04	0.002	0.84±0.05	0.10
Urine IL-18	0.65±0.07	0.04	0.009	0.85±0.05	0.89
Urine KIM-1	0.63±0.06	0.07	0.007	0.86±0.05	0.79
Uromodulin	0.54±0.07	0.54	0.002	0.85±0.05	0.94
Urine IGFBP-7	0.62±0.09	0.20	<0.001	0.88±0.05	0.57
Urine TIMP-2	0.70±0.08	0.03	0.02	0.83±0.06	0.20
Urine IGFBP-7×TIMP-2	0.69±0.08	0.04	0.01	0.90±0.06	0.35
Urine Creatinine	0.48±0.08	0.77	<0.001	0.84±0.06	0.85
Urine ACR	0.56±0.07	0.45	0.002	0.84±0.06	0.32
FeNa	0.51±0.07	0.92	<0.001	0.83±0.06	0.47
Plasma NGAL	0.75±0.08	0.007	0.10	0.86±0.07	0.53

NA, not applicable; ACR, albumin-to-creatinine ratio.

Table 3. AUCs for prediction of receipt of inpatient RRT

Biomarker	AUC±SEM	*P* Value for Biomarker Alone	*P* Value Compared With FST alone	AUC of Biomarker and FST±SEM	*P* Value for Biomarker and FST Compared With FST Alone
FST (2-hr UOP)	0.86±0.08	0.0001	NA	NA	NA
Urine NGAL	0.50±0.08	0.96	0.0006	0.88±0.06	0.35
Urine IL-18	0.61±0.07	0.26	0.03	0.85±0.09	0.70
Urine KIM-1	0.61±0.10	0.27	0.05	0.85±0.09	0.77
Uromodulin	0.55±0.11	0.60	0.07	0.89±0.06	0.65
Urine IGFBP-7	0.57±0.12	0.61	0.05	0.90±0.06	0.29
Urine TIMP-2	0.62±0.12	0.33	0.17	0.83±0.09	0.57
Urine IGFBP-7×TIMP-2	0.61±0.13	0.37	0.10	0.89±0.07	0.23
Urine creatinine	0.64±0.11	0.19	0.24	0.84±0.09	0.90
Urine ACR	0.67±0.09	0.13	0.28	0.86±0.08	0.51
FeNa	0.64±0.09	0.18	0.11	0.85±0.09	0.27
Plasma NGAL	0.52±0.13	0.88	0.07	0.80±0.13	0.92

NA, not applicable; ACR, albumin-to-creatinine ratio.

test, computed tomography, or ventilation-perfusion scanning for pulmonary embolus). While the number of these "high-risk" patients was low, these analyses demonstrate a potential use for the selective combination of biochemical biomarkers and FST (Table 6). The improved risk stratification achieved from biomarker cutoffs is in contrast to the analyses in which biomarker data from all patients is used (Tables 2–5) and merits further investigation in larger prospective cohorts.

To our knowledge, this study is the first to compare a diuretic challenge and biomarkers in patients with AKI. However, our investigation builds on existing literature that similarly demonstrates response to diuretics in the setting of decompensated heart failure predicts adverse patient outcomes.[14] While physicians have been informally using some form of the FST in a variety of clinical settings for decades, our standardization of this renal stress test has permitted a formal evaluation of the outcomes and comparisons with biomarkers. Importantly, before receiving the FST all patients in this study were clinically evaluated as euvolemic and stable for furosemide challenge, as determined by their treating physicians, before receiving this intervention. In addition, intravenous fluids to provide volume replacement for FST-induced urinary losses were permissible for euvolemic patients at the discretion of the treating team (see Concise Methods).[13]

Our data demonstrate the promise of FST in improving risk stratification of patients with early AKI. Previously, several biomarker studies investigated the utility of biomarkers of AKI to predict AKI progression in those with the earliest stages of AKI.[8–11] The Translational Research Investigating Biomarker Endpoints in AKI (TRIBE AKI) published biomarker data on 380 adults who developed AKIN stage 1 AKI after cardiac surgery.[9] Among these patients with AKI defined by changes in serum creatinine, 45 (11.8%) developed progressive AKI and 10 (22%) died during the postoperative hospital stay. TRIBE AKI demonstrated that albumin-to-creatinine ratio, urinary IL-18, and plasma NGAL predicted progression to AKIN stage 3 after adjustment for clinical factors known to be associated with severe AKI (AUC, ±SEM 0.78±0.04,

Table 4. AUCs for prediction of inpatient mortality

Biomarker	AUC±SEM	*P* Value for Biomarker Alone	*P* Value Compared With FST alone	AUC of Biomarker and FST±SEM	*P* Value for Biomarker and FST Compared With FST Alone
FST (2-hr UOP)	0.70±0.09	0.02	NA	NA	NA
Urine NGAL	0.66±0.08	0.06	0.82	0.71±0.08	0.64
Urine IL-18	0.57±0.09	0.40	0.30	0.63±0.09	0.55
Urine KIM-1	0.68±0.07	0.04	0.90	0.70±0.08	0.81
Uromodulin	0.52±0.08	0.79	0.18	0.65±0.08	0.71
Urine IGFBP-7	0.65±0.11	0.19	0.48	0.66±0.11	0.45
Urine TIMP-2	0.58±0.12	0.47	0.78	0.62±0.12	0.60
Urine IGFBP-7×TIMP-2	0.64±0.11	0.22	0.46	0.66±0.12	0.36
Urine creatinine	0.42±0.07	0.37	0.38	0.65±0.07	0.75
Urine ACR	0.47±0.09	0.68	0.10	0.61±0.10	0.46
FeNa	0.41±0.08	0.31	0.04	0.66±0.08	0.76
Plasma NGAL	0.43±0.11	0.55	0.34	0.56±0.11	0.94

NA, not applicable; ACR, albumin-to-creatinine ratio.

Table 5. Prediction of the composite of AKIN stage 3 and death

Biomarker	AUC±SEM	*P* Value for Biomarker Alone	*P* Value Compared With FST alone	AUC of Biomarker and FST±SEM	*P* Value for Biomarker and FST Compared With FST Alone
FST (2-hr UOP)	0.81±0.06	<0.0001	NA	NA	NA
Urine NGAL	0.69±0.06	0.006	0.07	0.82±0.06	0.89
Urine IL-18	0.63±0.07	0.07	0.009	0.82±0.06	0.87
Urine KIM-1	0.64±0.06	0.04	0.04	0.82±0.06	0.81
Uromodulin	0.54±0.07	0.58	0.004	0.85±0.06	0.31
Urine IGFBP-7	0.65±0.08	0.07	0.19	0.79±0.08	0.80
Urine TIMP-2	0.66±0.08	0.06	0.18	0.80±0.08	0.75
Urine IGFBP-7×TIMP-2	0.68±0.08	0.03	0.27	0.78±0.08	0.93
Urine Creatinine	0.54±0.07	0.56	0.007	0.83±0.06	0.23
Urine ACR	0.50±0.07	0.96	0.002	0.82±0.06	0.32
FeNa	0.49±0.07	0.84	0.009	0.80±0.06	0.31
Plasma NGAL	0.69±0.08	0.03	0.27	0.80±0.08	0.76

NA, not applicable; ACR, albumin-to-creatinine ratio.

Table 6. Prediction of patient outcomes by FST in patients with elevated biomarker levels

Variable	Urine NGAL>150 ng/ml (*n*=44)			Urine TIMP-2×IGFBP-7>0.3 (*n*=32)		
	Patients, *n* (%)	AUC±SEM	*P* Value	Patients, *n*	AUC±SEM	*P* Value
Progression to AKIN stage 3	19 (43.2)	0.86±(0.06	<0.001	11 (34.4)	0.90±0.06	<0.001
Receipt of inpatient RRT	7 (15.9)	0.91±0.06	<0.001	4 (12.5)	0.91±0.08	0.009
Inpatient death	12 (27.3)	0.72±(0.10	0.03	5 (15.6)	0.53±0.19	0.85
Progression to AKIN stage 3 or inpatient death	24 (54.5)	0.89±0.06	<0.001	14 (43.8)	0.81±0.10	0.003

0.77±0.04, and 0.8±0.04, respectively). Using this same clinical model, a plasma NGAL>322 ng/ml predicted a 7.7-fold increased risk of AKI progression. Similarly, the Southern AKI Network (SAKInet) measured 32 candidate biomarkers in the urine of 95 adults who developed AKIN stage 1. In this study, 23 patients (24.2%) developed worsened AKI or died while 13 (13.7%) met the secondary end point of AKIN stage 3 or death. In the similarly sized study, urinary IL-18 provided an AUC of 0.89 (95% confidence interval, 0.75 to 0.95) for this secondary end point, with IL-6, cystatin C, KIM-1, and NGAL all providing AUCs >0.80.[11] Similarly, Kashani *et al.* recently demonstrated that in a cohort of 744 critically ill patients (mixed medical and surgical intensive care unit [ICU]), some with Kidney Disease Improving Global Outcomes (KDIGO) stage 1 AKI and some with no AKI, TIMP2×IGFBP-7 measured early in the ICU course (at study enrollment) predicted who would develop KDIGO stage 2 or 3 AKI within the subsequent 12 hours (AUC, 0.80). When these biomarkers were analyzed separately

for AKI severity, IGFBP-7 and TIMP-2 exhibited an AUC of 0.76 and 0.79, respectively.[10]

Of note, differences between these studies and our pilot investigation may explain the discrepancy in biochemical biomarker performance. Our pilot cohort contained patients with AKIN stage 2 AKI. While we attempted to preferentially enroll patients who had AKIN stage 1, the consent process for most patients meant they were enrolled and received the FST 6–12 hours after the clinical criteria for stage 1 had been established. By that time several patients had already progressed to stage 2. This heterogeneity of AKI severity affects biomarker performance.[15,16] The analyses from the TRIBE AKI,[9] SAKInet,[11] and Kashani[10] *et al.* cohorts all excluded patients with stage 2 AKI at the time of biomarker measurement. In addition to the slightly increased severity of AKI in our cohort, the timing of our biomarker measurements was slightly different from that in the TRIBE-AKI and SAKInet protocols. Given the time lag involved in identifying and consenting patients for the FST protocol, biomarkers were often measured 6–12 hours after clinical evidence of AKI was determined. Thus, it is not surprising that injury or structural biomarkers did not perform well in this cohort because the kinetics of these biomarkers are not suited for detection at this delayed timepoint in this established AKI population.[6,17,18] Despite the poor performance of some biochemical biomarkers in our pilot data, the aforementioned studies provide ample evidence to support the continued investigation of biomarkers at the time of AKI for the prediction of progression, as well as a role for investigation of biomarkers of renal recovery. We expect that when FST is investigated in a larger cohort and is used in combination with biomarkers of AKI, these indicators of renal tubule integrity will synergize and further improve risk stratification for AKI severity and other adverse patient outcomes.

As clinicians move into an era of AKI prophylactic and therapeutic trials we need safe, reliable and standardized methods to predict AKI progression.[5,19–21] Currently we lack the tools to reliably determine which patients with early AKI (or no AKI) will progress to severe outcomes, such as the need for RRT or death. In our study 32.5% of those with early AKI progressed to stage 3 AKI while 27.3% received RRT or died. Thus, for every 3–4 patients we enrolled, only 1 experienced a hard clinical end point. In an AKI interventional trial a screening tool such as FST (or FST combined with biomarkers) may provide investigators resource and financial efficiency. Maximizing enrollment of patients with AKI who will complete a clinically meaningful end point (most severe stages of AKI, receipt of dialysis, or inpatient death) will be important for clinical trialists, and the FST is potentially an optimal tool for this task.[22]

Our study has several strengths. We relied on standardized AKI staging criteria (AKIN) that are internationally accepted and can be readily duplicated in future investigations. Similarly, we used hard clinical endpoints, such as receipt of RRT or inpatient death, that can also be duplicated. We have compared the FST to eight of the most widely investigated AKI biomarkers, including many of those that have shown promise for the prediction of AKI progression.[9–11] In addition, those running the biologic assays were blinded to patient outcomes.

However, given the size of our pilot cohort and the limited number of patient events, we are not powered to extend our multivariate modeling beyond the simple investigations of FST-biomarker combinations presented. Thus, our ability to analyze the combinations of biochemical biomarkers, without FST, to forecast the development of adverse patient outcomes was limited. Given the time-lag that is inherent to conducting clinical research in the setting of clinical illness, however, measuring damage or injury biomarkers 6–12 hours after clinical AKI may not be an appropriate comparison. Moreover, extrapolation of our data to multivariable models, as presented in our original pilot data, would not be statistically appropriate given our cohort size and the number of biomarkers we investigated. However, we have previously shown that FST remains a significant predictor of AKI progression even after controlling for pre-FST urine output, GWU-USS cast score, change in serum creatinine, and prior furosemide exposure.[13] Finally, we are limited by the lack of follow-up after discharge for those who survived their FST index hospitalization.

In summary, in patients with early AKI stable enough to undergo FST, 2-hour urine output serves as a promising tool for assessment of AKI severity and prognosis. As a functional biomarker of AKI, FST was superior to the panel of biochemical biomarkers for all end points. Improving risk prediction in those with early AKI is likely to alter patient care and clinical decision making, as well as facilitate enrollment into future therapeutic AKI trials. Our pilot study did not demonstrate statistically improved risk prediction when FST was combined with biochemical biomarkers of AKI. This may have been a limitation of our sample size and the timing of biomarker measurement. Given the wealth of data supporting the potential utility of biomarkers of AKI, larger prospective validations of the FST, with biomarkers, should be conducted.

CONCISE METHODS

As previously described,[13] we assembled two separate cohorts of critically ill patients with stage 1 or 2 AKIN criteria[11,23–25] who were given a standardized dose of furosemide and had their urinary response and inpatient outcomes assessed. Briefly, our study combines patients who were part of the SAKInet cohort at George Washington University and were retrospectively identified as having received an FST,[11,23,24] and patients who had their FST prospectively from June 2009 through December 2012 at George Washington University (NCT00673244) or the University of Chicago (NCT01275729).

Study Criteria

To receive the FST, patients had to meet the following inclusion criteria*:* (*1*) age older than 18 years and admission in an ICU; (*2*) AKIN stage 1 (6 hours of oliguria [<0.5 ml/kg per hour] or 0.3-mg/dl rise in serum creatinine or 50%–100% increase in serum creatinine

above baseline) or AKIN stage 2 (12 hours of oliguria [<0.5 ml/kg per hour] or 100%–200% increase in serum creatinine above baseline during a 48-hour period); (*3*) indwelling bladder catheter; (*4*) presence of granular or epithelial cell casts on urine sediment (defined by GWU-USS score ≥2)[26] or a FeNa > 1.0%; and (*5*) opinion of the treating clinical team that patient was well resuscitated and sufficiently clinically stable for the intervention.

Patients were excluded if they met any of the following exclusion criteria: (*1*) baseline eGFR<30 ml/min per 1.73 m^2, (*2*) history of renal allograft, (*3*) known pregnancy, (*4*) evidence of obstructive uropathy, (*5*) evidence of active bleeding, (*6*) allergy or known sensitivity to loop diuretics, (*7*) achievement of AKIN stage 3 criteria, or (*8*) evidence of volume depletion at the time of furosemide administration and (*9*) prior episode of AKIN stage 1 or 2 during the same hospital admission.

The study procedures for the administration of the FST (furosemide dose of 1.0 mg/kg or 1.5 mg/kg) and the option for intravenous fluid replacement matching urine output in the 6 hours after FST have all been previously described.[13] All ICU patients were being screened for eligibility on a daily basis. Once a patient was identified, the investigator research team approached the clinical treating service to determine whether the patient was hemodynamically appropriate and sufficiently clinically stable to undergo an FST. Inherent in the assessment for FST is that the patient was not undergoing active volume expansion or active resuscitation. In addition, for patients who had previously been resuscitated, readiness for FST implied that the patient was no longer hypovolemic or under-resuscitated. The treating team had the final determination regarding the patient's ability to receive the FST and was presented with the option of real-time replacement of urinary losses with isotonic intravenous fluid. Once the treating team approved, the patient (or proxy) was approached for informed consent. Informed consent was often obtained 6–12 hours after the patient met AKIN stage 1 criteria. This time delay explains how patients with AKIN stage 2 were enrolled. Additionally, if a patient was not naive to furosemide (or other diuretic), the FST was not administered until at least 6 hours after the prior diuretic dose.

We defined the baseline creatinine as the nadir outpatient serum creatinine from the 6 months before the admission. If no serum creatinine values from before the admission were available, then the baseline serum creatinine was defined as the lowest serum creatinine from this admission.

Outcomes

The primary outcome was the progression to AKIN stage III (need for RRT, increase in serum creatinine of 300% over baseline, a serum creatinine level>4.0 mg/dl with an acute rise of at least 0.5 mg/dl, urine output of 0.3 ml/kg per hour×24 hours or anuria for 12 hours) within 14 days of FST. The secondary outcomes included inpatient mortality and the receipt of RRT.

Biomarker Assays

All biomarker samples and measures were collected just before the administration of furosemide. TIMP-2 and IGFBP-7 were measured by Astute Medical Inc. as previously described.[10,12] Plasma and urine NGAL were measured using a commercially available ELISA (Bioporto, Hellerup, Denmark) in the Biomarker Lab in the Section of Nephrology and Hypertension at the Cincinnati Children's Hospital. Urinary IL-18 was measured at Cincinnati Children's Hospital using an ELISA (MBL International, Woburn, MA) as previously described.[9,17,27] Urine creatinine concentration was measured using the Jaffe method on a Siemens Dimension Xpand Plus HM clinical analyzer. Urinary KIM-1 was measured at Cincinnati Children's Hospital using a previously described ELISA (intra-assay coefficient of variation, 5.5%; interassay coefficient of variation, 5.0%) (Covance, Central Laboratory Services, Indianapolis, IN).[28] Uromodulin was measured at Cincinnati Children's Hospital using an ELISA (MD Biosciences, St. Paul, MN) (intra-assay coefficient of variation, 8.5%; interassay coefficient of variation, 11%). Urine albumin and sodium assays were measured by immunoturbidimetry on a Siemens Dimension Plus with a Heterogeneous Immunoassay clinical analyzer (Siemens Healthcare Diagnostics, Deerfield, Ill), as previously described.[29] All laboratory personnel were blinded to patient outcomes during the measurement of the biomarkers.

Statistical Analyses

We assessed the distribution of demographic and clinical variables. Differences between proportions of patients with certain characteristics were assessed with chi-squared tests, Fisher exact test, *t*-tests, and Mann–Whitney test as appropriate. The primary analysis was to compare the ability of urine output response to the FST and biomarkers to predict the primary end point of progression to AKIN stage 3 and the secondary end points of death and receipt of RRT within 14 days of the FST, determined by assessing the AUC. The AUCs were compared using the method described by DeLong *et al.*[30] and, when appropriate, a Bonferroni adjustment was made to account for multiple comparisons. Additionally, we used logistic regression models consisting of FST and the individual biomarkers and evaluated the discriminatory ability of the combinations to predict patient outcomes compared with the FST alone. Finally, we performed a sequential analysis using biochemical biomarkers and FST, where we first used biomarker cutoffs provided by pre-existing literature for NGAL (>150 ng/ml) and TIMP-2/IGFBP-7 (>0.3). For patients whose biomarker concentration exceeded the cutoff, we then assessed the AUC for FST for all patient outcomes.[12,31] We performed all analyses for urinary biomarkers unadjusted (data below) and adjusted for urinary creatinine concentration (data not shown). There was no significant difference between the two.

All means are reported as ±SEM unless otherwise specified. Statistical analysis was performed using SPSS software, version 18.0 (SPSS Inc., Chicago, IL) and Stata 13.0 (StataCorp., College Station, TX). Methods used to calculate FeNa, Acute Physiology and Chronic Health Evaluation II score, and cardiovascular Sequential Organ Failure Assessment score and eGFR were previously described.[13]

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DISCLOSURES

J.L.K. reports consulting fees from Astute Medical Inc. and payments for enrolling patients in observational biomarker studies from Astute and Abbott. A.D.S., reports consulting fees from Astute Medical Inc. L.S.C. reports links to Alere Medical and Astute Medical Inc.

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See related editorial, "The Furosemide Stress Test and Predicting AKI Outcomes," on pages 1762–1764.