Urine Biomarkers Predict Acute Kidney Injury in Newborns

David J. Askenazi, MD, MSPH[1], Rajesh Koralkar, MPH[2], Hayden E. Hundley, MPH[3], Angela Montesanti, MPH[4], Pushkar Parwar, MBBS, MPH[1], Srdjan Sonjara, BS, BA[5], and Namasivayam Ambalavanan, MD[1]

Objective To identify urine biomarkers predictive of acute kidney injury (AKI) in infants admitted to level 2 and 3 neonatal intensive care units with birth weight >2000 g and 5-minute Apgar score ≤7.

Study design A nested case-control study was performed comparing 8 candidate urine AKI biomarkers in infants with AKI (defined as a rise in serum creatinine of at least 0.3 mg/dL or a serum creatinine elevation ≥1.7 mg/dL persisting for 3 days) and 24 infants from the described cohort without AKI. Urine was analyzed for neutrophil gelatinase–associated lipocalin, osteopontin, cystatin C, albumin, β_2 microglobulin, epithelial growth factor, uromodulin (UMOD), and kidney injury molecule 1.

Results Compared with the infants without AKI, those with AKI had higher levels of urine cystatin C (1123 pg/mL [95% CI, 272-4635 pg/mL] vs 90 pg/mL [95% CI, 39-205 pg/mL]; $P < .004$; area under the receiver operating characteristic curve [AUC] = 0.82), lower levels of UMOD (11.0 pg/mL [95% CI, 5.7-21.4 pg/mL] vs 26.2 pg/mL [95% CI, 17.4-39.4 pg/mL]; $P < .03$; AUC = 0.77), and lower levels of epithelial growth factor (6.7 pg/mL [95% CI, 4.0-11.3 pg/mL] vs 17.4 pg/mL [95% CI, 12.7-23.8 pg/mL; $P = .003$; AUC = 0.82). Although the differences were not statistically significant, levels of urine neutrophil–associated gelatinase lipocalin, kidney injury molecule 1, and osteopontin trended higher in infants with AKI.

Conclusion Urinary biomarkers can predict AKI in neonates admitted to level 2 and 3 neonatal intensive care units. *(J Pediatr 2012;161:270-5)*.

The reported incidence of acute kidney injury (AKI) in neonates with a 5-minute Apgar score <7 ranges from 47% to 61%.[1-3] AKI has been shown to be an independent predictor of mortality in critically ill neonates,[4,5] children,[6,7] and adults[8-12] even after controlling for comorbidities, interventions, and demographic data. AKI not only impairs fluid and electrolyte homeostasis, but also may hamper systemic inflammatory autoregulation.[13] The injured kidneys may play a key role in the systemic derangement present during multiorgan failure. Given that a rise in serum creatinine (SCr) level usually does not occur until several days after renal injury,[14] the search for early AKI biomarkers has assumed a prominent role in advancement of AKI research.

SCr-based definitions are used to diagnose AKI.[15,16] SCr-based definitions are not ideal, however, because (1) SCr measures function, not injury; (2) SCr may not change until 25%-50% of kidney function has already been lost; (3) SCr overestimates renal function due to tubular secretion of creatinine at lower glomerular filtration rates; (4) SCr varies by muscle mass, hydration status, sex, and age, and its measurement can be affected by endogenous substances; (5) SCr cannot distinguish between prerenal azotemia (a transient, reversible decrease in glomerular filtration rate), and bona fide kidney injury; (6) SCr is not specific to different types of AKI (nephrotoxic, sepsis-associated, or hypoxic/ischemic); and (7) SCr cannot be used to assess kidney function while a patient is receiving dialysis.[17] Other problems with using SCr as a measure of AKI specific to neonates include the fact that SCr in the first few days of life reflects maternal kidney function, and there is a very wide distribution of normal SCr values that change over time, depending on the level of prematurity.[18,19]

Urine AKI biomarkers have shown to be predictive of AKI and mortality in children undergoing cardiopulmonary bypass[20-25] and in critically ill preterm infants.[26] To date, only limited evaluations of biomarkers have been performed in neonates. To investigate the utility of urine AKI biomarkers in neonates, we evaluated 8 previously identified can-

AKI	Acute kidney injury
AUC	Area under the receiver operating characteristic curve
B2mG	β_2 microglobulin
Cys C	Cystatin C
CV	Confidence of variability
EGF	Epithelial growth factor
KIM-1	Kidney injury molecule 1
NGAL	Neutrophil gelatinase–associated lipocalin
OPN	Osteopontin
SCr	Serum creatinine
UMOD	Uromodulin

From the Departments of [1]Pediatrics and [2]Public Health, University of Alabama at Birmingham, Birmingham, AL; [3]Department of Medicine, University of South Alabama College of Medicine, Mobile, AL; [4]Center for Healthy Development, Georgia State University Institute of Public Health, Atlanta, GA; and [5]O'Brien Center for AKI Research, University of California at San Diego, La Jolla, CA

Supported by a The Normal Siegel Scholar Young Investigator Award from the American Society of Nephrology. (to D.A.), the Kaul Pediatric Research Institute, and a pilot and feasibility grant from the National Institutes of Health–sponsored O'Brien Center for Acute Kidney Injury research (www.obrienaki.org). D.A. serves as consultant and is on the speaker's bureau for Gambro Renal Products. The other authors declare no conflicts of interest.

 10.1016/j.jpeds.2012.02.007

didate urinary biomarkers: neutrophil gelatinase–associated lipocalin (NGAL), osteopontin (OPN), cystatin C (Cys C), albumin, β_2 microglobulin (B2mG), epithelial growth factor (EGF), uromodulin (UMOD), and kidney injury molecule 1 (KIM-1). We explored the individual and combined ability of these biomarkers to predict AKI. Because urinary proteins are reabsorbed poorly in the most preterm infants,[27-29] we performed regression analysis for gestational age to ensure that elevation of biomarkers were not due solely to immature tubular function.

Methods

We conducted a nested case-control study to evaluate the ability of 8 urine biomarkers to predict AKI. Nested case-control studies are particularly advantageous for studies of biologic precursors of disease, such as those described in this study.[30]

Subjects were newborns in the regional neonatal intensive care unit of the University of Alabama at Birmingham between January 2010 and March 2011. The inclusion criteria for eligibility were birth weight >2000 g, gestational age >34 weeks, 5-minute Apgar score ≤7, and parental informed consent. Of the 130 infants who met the inclusion criteria, 1 infant was excluded for congenital anomaly of the kidney, parents of 71 infants did not consent to participate (35 not interested, 30 not available, 4 infants were transferred to another unit, 2 unknown), and parents of 58 infants consented. Nine of these infants met the criteria for AKI. To serve as controls, we chose 24 infants with ample SCr samples to allow confirmation of negative AKI status (**Figure 1**; available at www.jpeds.com). The University of Alabama at Birmingham's Institutional Review Board approved the study.

AKI was defined as an acute rise in SCr of at least 0.3 mg/dL within 48 hours (stage 1 of the AKI Network definition[16]) or a persistent rise in SCr to ≥1.7 mg/dL for 3 days after birth. Controls comprised infants who met the inclusion criteria and had at least 2 blood samples to confirm negative AKI status. SCr values were measured by the Jaffe reaction and obtained during the first 4 days of life. The average number of SCr measurements, infant demographic data, infant interventions, and maternal characteristics in those with AKI and without AKI are presented in **Table I**.

Urine specimens were collected during the first 4 days of life using cotton balls placed at the perineum. Not all infants had urine collected on all days. The average number of urine samples obtained in cases was 2 (range, 1-3), and the average for controls was 2 (range, 2-3). Urine was extracted, centrifuged for 10 minutes, and then frozen at −80°C until evaluation. Urine biomarker analysis was performed at Core A of the National Institutes of Health's P30 O'Brien Core Center for AKI research (www.obrienaki.org).

The Meso Scale Discovery Human Kidney Injury Panel-5 Prototype 7-Plex Assay Kit (catalog no. N75CA-1; Meso Scale Discovery, Gaithersburg, Maryland) was used to measure OPN, Cys C, NGAL, albumin, B2mG, EGF, and UMOD concentrations in 91 human urine samples. Good reproducibility of standard duplicates was obtained, with an average signal confidence of variability (CV) of 6.5%. For 24 samples measured in duplicate, good reproducibility was observed, with an average signal CV of 6.1% and an average calculated concentration CV of 7.04%. The Human Kidney Injury Panel-5 7-Plex assay has picogram per milliliter sensitivity and covers a broad concentration range, from low pg/mL up to 200 000 pg/mL. Urine samples diluted 1:100 yielded good values for Cys C, NGAL, OPN, EGF, and UMOD. At a 1:100 dilution, several albumin and B2mG values were above the curve fit range.

The Meso Scale Discovery Human KIM-1 Assay Kit (catalog no. N45ZA-1; Meso Scale Discovery, Gaithersburg, Maryland) was used to estimate KIM-1 concentrations in 91 human urine samples. Excellent reproducibility of standard duplicates was obtained, with an average signal CV of 2.5%. For 24 samples measured in duplicate, good reproducibility was seen, with an average signal CV of 3.5% and an average calculated concentration CV of 6.2%. The human KIM-1 assay covers a broad concentration range, from <7 pg/mL up to >100 000 pg/mL, allowing for measurement

Table I. Demographic data for infants with AKI and those without AKI

	No AKI (n = 24)	AKI (n = 9)	*P* value
Infant characteristics			
Male sex, n (%)	9 (38)	8 (89)	<.01
Race, n (%)			.50
Black	13 (54)	4 (44)	
White	9 (38)	5 (56)	
Hispanic	2 (8)	0 (0)	
Gestational age, weeks, mean ± SD	35 ± 3	37 ± 3	.10
Birth weight, g, mean ± SD	2421 ± 631	3425 ± 863	<.001
Length, cm, mean ± SD	46.4 ± 3.6	49.2 ± 5.0	.87
Head circumference, cm, mean ± SD	31.5 ± 2.1	33.4 ± 1.8	<.05
Survival, n (%)	24 (100)	7 (77)	.07
Number of urine collections, mean ± SD	2 (1-3)	2 (1-3)	NS
1-minute Apgar, median (IQR)	2 (1-6)	1 (0-5)	.10
5-minute Apgar, median (IQR)	7 (4-7)	5 (0-7)	.06
Cord PH, mean ± SD	7.14 ± 0.15	6.97 ± 0.21	<.01
Infant interventions			
Delivery room bag, n (%)	7 (29)	3 (33)	.56
Delivery room oxygen, n (%)	23 (95)	8 (88)	.47
Delivery room pressors, n (%)	20 (83)	7 (77)	.53
Delivery room intubation, n (%)	4 (16)	5 (55)	<.05
Surfactant, n (%)	2 (8)	2 (22)	.30
Phenobarbitol, n (%)	4 (16)	1 (11)	<.01
Pressors during hospital, n (%)	5 (20)	1 (11)	<.01
Aminoglycoside, n (%)	21 (88)	7 (78)	.60
Standard ventilator, n (%)	18 (75)	6 (67)	.70
Maternal characteristics			
Age, mean ± SD	25.6 ± 6.0	23.2 ± 4.8	.30
Hypertension, n (%)	6 (25)	1 (11)	.36
Preeclampsia, n (%)	8 (33)	0 (0)	.07
Smoking, n (%)	6 (25)	2 (22)	.62
Antibiotic, n (%)	16 (66)	6 (66)	.65
Diabetes mellitus, n (%)	5 (20)	3 (33)	.37
Chorioamnionitis, n (%)	3 (12)	0 (0)	.37
Steroids, n (%)	7 (29)	2 (22)	.52
Prenatal care, n (%)	23 (95)	8 (88)	.47