Urine Biomarkers Predict Acute Kidney Injury and Mortality in Very Low Birth Weight Infants

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Objectives To test the hypothesis that noninvasive urinary biomarkers may improve early identification, differentiate causes, and predict outcomes of acute kidney injury (AKI) in very low birth weight subjects.

Study design We performed 2 nested case-control studies to compare the ability of 6 urine biomarkers to predict AKI (rise in serum creatinine of at least 0.3 mg/dL) and mortality (death before 36 weeks postmenstrual age).

Results Compared to subjects without AKI (n = 21), those with AKI (n = 9) had higher maximum neutrophil gelatinase-associated lipocalin (OR = 1.2 [1.0, 1.6]; $P < .01$; receiver operator characteristics [ROC] area under the curve [AUC] = .80) and higher maximum osteopontin (OR = 3.2 [1.5, 9.9]; $P < .01$; ROC AUC = 0.83). Compared with survivors (n = 100), nonsurvivors (n = 23) had higher maximum kidney injury molecule 1 (OR = 1.1 [1.0, 1.2]; $P < .02$; ROC AUC = 0.64) and higher maximum osteopontin (OR = 1.8 (1.2, 2.7); $P < .001$; AUC of ROC = 0.78). The combination of biomarkers improved predictability for both AKI and mortality. Controlling for gestational age and birth weight did not affect results considerably.

Conclusions Urinary biomarkers can predict AKI and mortality in very low birth weight infants independent of gestational age and birth weight. *(J Pediatr 2011;159:907-12)*.

Morbidity and mortality remain high in premature infants.[1,2] Acute kidney injury (AKI), previously referred to as acute renal failure, is common in premature infants.[3,4] AKI is 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 demographics. AKI not only impairs fluid and electrolyte homeostasis but may also hamper systemic inflammatory autoregulation,[13] AKI may play a key role in the systemic derangement present during multiorgan failure. Therapies known to prevent or ameliorate AKI in animal models have not shown reduction in clinical AKI studies. One of the possible reasons for these negative results is that the interventions were instituted only after a rise in serum creatinine (SCr). It usually take days after renal injury for a rise in SCr to occur,[14] so the search for early AKI biomarkers has taken a prominent role in AKI research.

Currently, SCr-based definitions are used to diagnose AKI.[15,16] However, SCr-based definitions are not ideal for the following reasons[3]: (1) SCr measures function, not injury; (2) SCr may not change until 25% to 50% of the 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, age, medications, and endogenous substances like bilirubin; (5) SCr can be nonspecific for AKI, especially with prerenal azotemia (a transient, reversible decrease in glomerular filtration rates); and (6) SCr cannot be used to assess kidney function while patients are receiving dialysis. Additional problems in neonates are that SCr level during the first few days of life reflects the mother's, not the infant's kidney function. There is a very wide distribution of normal SCr values that change over time, depending on the level of prematurity.[17,18]

To determine whether urine biomarkers can be used to detect AKI in very low birth weight (VLBW) infants, we evaluated 6 previously identified candidates for urinary biomarkers: neutrophil gelatinase-associated lipocalin (NGAL); interleukin-18 (IL-18); kidney injury molecule-1 (KIM-1); osteopontin (OPN); beta-2 microglobulin (B2mG)[19,20]; and cystatin-C (Cys-C). We explored the individual and combined abilities of these biomarkers to predict

AKI	Acute kidney injury
AUC	Area under the curve
B2mG	Beta-2 microglobulin
Cys-C	Cystatin-C
IL-18	Interleukin-18
KIM-1	Kidney injury molecule-1
NGAL	Neutrophil gelatinase associated lipocalin
OPN	Osteopontin
ROC	Receiver operator characteristics
SCr	Serum creatinine
VLBW	Very low birth weight

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AKI and also their ability to predict mortality. Because some of these biomarkers may vary by gestational age and birth weight,[21-23] we performed regression analysis to control for these potential confounders.

Methods

We conducted 2 separate nested case-control studies to determine the ability of 6 urine biomarkers to predict AKI and mortality. In a nested case-control study, data were collected prospectively. Using these data, patients who have the disease (AKI, for example) that occurs in the defined cohort are identified. Then controls (those who do not have the disease [the no-AKI group]) are selected from the prospective cohort. The advantage of this design is that a nested case-control design potentially offers reductions in costs and efforts of data collection compared with the full-cohort approach, and there is a relatively minor loss in statistical efficiency. It is particularly advantageous for studies of biologic precursors of disease such as those described in this article.[24]

The original cohort comprised VLBW infants (birth weight range of 500 g to 1500 g) who had been admitted to the regional quaternary care neonatal intensive care units of the University of Alabama at Birmingham and the Children's Hospital of Alabama between February 2008 and July 2009. Infants were excluded if they did not survive to 48 hours of life or if they had any significant known congenital abnormality of the kidney. The incidence of AKI and association with outcomes in this cohort has been described.[4] Parental consent was obtained, and the Institutional Review Board at the University of Alabama at Birmingham approved the study.

Populations Used to Evaluate the Ability of Biomarkers to Detect Acute Kidney Injury

Cases include VLBW infants who experienced AKI, which is defined as an acute rise in SCr of at least 0.3 mg/dL within 48 hours (stage 1 of the Acute Kidney Injury Network's definition).[15] Controls consisted of VLBW infants who did not have AKI but had ample blood samples to confirm negative AKI status around the time of urine-sample collection. Serum was analyzed from remnant serum samples when available and from the patients' medical records when the sample was taken as part of routine hospital care. Table I compares infant and maternal characteristics of those with and without AKI.

Population Used to Evaluate the Ability of Biomarkers to Predict Mortality

Cases (nonsurvivors) were infants who did not survive to 36 weeks postmenstrual age. Controls (survivors) were infants who were discharged home or survived to 36 weeks postmenstrual age. Hospital discharge or survival to 36 weeks postmenstrual age was used to define survival because most preterm infants' deaths occur within the first few weeks of life, and mortality subsequent to that time point is very unlikely.[25,26] Table II compares the demographic characteristics of infants and mothers in the groups of survivors and nonsurvivors.

Table I. Demographic differences between infants with and without AKI

	No AKI (n = 21)	AKI (n = 9)	*P* value
Infant Characteristics			
Birth weight (g)	958 ± 283	691 ± 200	<.01
Gestational age (wk)	27.7 ± 2.7	25.9 ± 2.8	.11
Sex			
Male	10 (47.6)	4 (44.4)	1.0
Female	11 (52.4)	5 (55.6)	
Race			
White	9 (42.9)	7 (77.8)	.12
Black	11 (47.8)	1 (11.1)	
Hispanic	1 (4.8)	1 (11.1)	
1-minute Apgar score*	5 (2, 7)	3 (1, 5)	.32
5-minute Apgar score*	7 (5, 8)	7 (5, 7)	.78
Vancomycin	14 (66.7)	8 (88.9)	.37
Aminoglycocide	20 (95.2)	8 (100)	1.0
Indomethacin	11 (52.4)	4 (44.4)	1.0
Umbilical catheter	11 (52.4)	7 (77.8)	.25
Maternal Characteristics			
Age (years)	28.5 ± 7.1	26.4 ± 5.5	.41
Hypertension	10 (47.6)	4 (44.4)	1.0

Continuous: mean ± SD (except * in which median [25%, 75% IQR]).
Categorical: n (%).

Biomarker Analysis

Urine was collected during the first 6 days of life by placing cotton balls at the perineum. Urine was extracted, centrifuged for 10 minutes to remove any cotton fibers or cellular elements, and then frozen at −70°C until sample evaluation. Urine biomarker analysis was performed by Core A of the National Institutes of Health P30 O'Brien Core Center for AKI research (www.obrienaki.org) using assays from Meso Scale Discovery (Gaithersburg, MD).

NGAL, Cys-C, OPN, and B2mG were measured in urine using a prototype 4-value multiplex (4-plex) assay. IL-18 and KIM-1 were measured using a prototype duplex

Table II. Demographic differences between survivors and nonsurvivors

	Survivors (n = 100)	Nonsurvivors (n = 23)	*P* value
Infant characteristics			
Birth weight (g)	952.2 ± 280.2	675.9 ± 230.01	<.0001
Gestational age (wks)	27.5 ± 0.25	25.3 ± 0.21	<.001
Sex			
Male	42 (75%)	14 (25%)	.10
Female	58 (86.6%)	9 (13.4%)	
Race			
White	39 (39.0%)	13 (56.5%)	.27
Black	53 (53.0%)	8 (37.8%)	
Hispanic	8 (8.0%)	2 (8.7%)	
1 minute Apgar Score*	4 (2, 7)	3 (1, 5)	.07
5 minute Apgar Score*	7 (6, 8)	6 (4, 7) 26%	<.03
Vancomycin	66 (66.0%)	21 (91.3%)	<.02
Aminoglycocide	95 (95%)	22 (96%)	.89
Indomethacin	50 (50%)	9 (39%)	.35
Umbilical Catheter	51 (51%)	16 (69.7%)	.10
AKI present	12 (16.2%)	15 (78.9%)	<.0001
Maternal Characteristics			
Age	26.1 ± 6.3	25.4 ± 5.1	.62
Hypertension	43 (43.0%)	5 (21.7%)	.06

Continuous: mean ± SD (except * in which median [25%, 75% IQR]).
Categorical: n (%).