Identification of Acute Kidney Injury Subphenotypes with Differing Molecular Signatures and Responses to Vasopressin Therapy

Pavan K. Bhatraju^{1,2}, Leila R. Zelnick², Jerald Herting³, Ronit Katz², Carmen Mikacenic¹, Susanna Kosamo¹, Eric D. Morrell¹, Cassianne Robinson-Cohen², Carolyn S. Calfee^{4,5,6}, Jason D. Christie^{7,8*}, Kathleen D. Liu^{9,10}, Michael A. Matthay^{4,5,6}, William O. Hahn¹¹, Victoria Dmyterko¹, Natalie S. J. Slivinski¹², Jim A. Russell^{13,14}, Keith R. Walley^{13,14}, David C. Christiani^{15,16,17}, W. Conrad Liles¹⁸, Jonathan Himmelfarb², and Mark M. Wurfel^{1,2}

¹Division of Pulmonary, Critical Care, and Sleep Medicine, ²Kidney Research Institute, Division of Nephrology, and ¹¹Division of Allergy and Infectious Diseases, Department of Medicine, ³Department of Sociology, and ¹⁸Department of Medicine, University of Washington, Seattle, Washington; ⁴Department of Medicine, ⁵Department of Anesthesia and Perioperative Care, ⁶Cardiovascular Research Institute, ⁹Division of Nephrology, and ¹⁰Division of Critical Care Medicine, University of California, San Francisco, San Francisco, California; ⁷Division of Pulmonary, Allergy, and Critical Care and ⁸Center for Clinical Epidemiology and Biostatistics, Department of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania; ¹²University of Leeds, Leeds, United Kingdom; ¹³Centre for Heart Lung Innovation and ¹⁴Division of Critical Care Medicine, Department of Medicine, St. Paul's Hospital, University of British Columbia, Vancouver, British Columbia, Canada; ¹⁵Department of Environmental Health and ¹⁶Department of Epidemiology, Harvard School of Public Health, Harvard University, Boston, Massachusetts; and ¹⁷Pulmonary and Critical Care Division, Department of Medicine, Massachusetts General Hospital/Harvard Medical School, Boston, Massachusetts

ORCID ID: 0000-0001-5151-9361 (S.K.).

Abstract

Rationale: Currently, no safe and effective pharmacologic interventions exist for acute kidney injury (AKI). One reason may be that heterogeneity exists within the AKI population, thereby hampering the identification of specific pathophysiologic pathways and therapeutic targets.

Objective: The aim of this study was to identify and test whether AKI subphenotypes have prognostic and therapeutic implications.

Methods: First, latent class analysis methodology was applied independently in two critically ill populations (discovery [$n = 794$] and replication [$n = 425$]) with AKI. Second, a parsimonious classification model was developed to identify AKI subphenotypes. Third, the classification model was applied to patients with AKI in VASST (Vasopressin and Septic Shock Trial; $n = 271$), and differences in treatment response were determined. In all three populations, AKI was defined using serum creatinine and urine output.

Measurements and Main Results: A two-subphenotype latent class analysis model had the best fit in both the discovery ($P = 0.004$) and replication ($P = 0.004$) AKI groups. The risk of 7-day renal

nonrecovery and 28-day mortality was greater with AKI subphenotype 2 (AKI-SP2) relative to AKI subphenotype 1 (AKI-SP1). The AKI subphenotypes discriminated risk for poor clinical outcomes better than the Kidney Disease: Improving Global Outcomes stages of AKI. A three-variable model that included markers of endothelial dysfunction and inflammation accurately determined subphenotype membership (C-statistic 0.92). In VASST, vasopressin compared with norepinephrine was associated with improved 90-day mortality in AKI-SP1 (27% vs. 46%, respectively; $P = 0.02$), but no significant difference was observed in AKI-SP2 (45% vs. 49%, respectively; $P = 0.99$) and the P value for interaction was 0.05.

Conclusions: This analysis identified two molecularly distinct AKI subphenotypes with different clinical outcomes and responses to vasopressin therapy. Identification of AKI subphenotypes could improve risk prognostication and may be useful for predictive enrichment in clinical trials.

Keywords: acute kidney injury; endothelial dysfunction; mortality; subphenotypes

(Received in original form July 20, 2018; accepted in final form October 12, 2018)

*J.D.C. is Associate Editor of *AJRCCM*. His participation complies with American Thoracic Society requirements for recusal from review and decisions for authored works.

Supported by grants from the NHLBI (R01HL060710); the National Institute of Diabetes and Digestive and Kidney Diseases (F32DK112532); and the Department of Medicine, University of Washington; and an unrestricted gift to the Kidney Research Institute from the Northwest Kidney Centers.

Author Contributions: Study concept and design: P.K.B., J. Herting, R.K., C.M., C.R.-C., W.C.L., J. Himmelfarb, and M.M.W. Acquisition, analysis, or interpretation of data: all authors. Drafting of the manuscript: P.K.B. Critical revision of the manuscript for important intellectual content: all authors. Statistical analysis: P.K.B., J. Herting, R.K., C.R.-C., and L.R.Z. Study supervision: J. Herting, J. Himmelfarb, W.C.L., and M.M.W.

Correspondence and requests for reprints should be addressed to Pavan K. Bhatraju, M.D., M.Sc., 325 9th Avenue, Seattle, WA 98104. E-mail: bhatraju@uw.edu.

This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org.

Am J Respir Crit Care Med Vol 199, Iss 7, pp 863–872, Apr 1, 2019

Copyright © 2019 by the American Thoracic Society

Originally Published in Press as DOI: 10.1164/rccm.201807-1346OC on October 18, 2018

Internet address: www.atsjournals.org

At a Glance Commentary

Scientific Knowledge on the

Subject: Acute kidney injury (AKI), as currently clinically defined, is caused by a heterogeneous set of pathophysiologic processes and is associated with variable outcomes. It is unknown whether this heterogeneity can be parsed into clinical subphenotypes with differing underlying biology, risk for clinical outcomes, and response to therapies.

What This Study Adds to the

Field: This study identified two novel AKI subphenotypes independently in two critically ill populations. To ease future subphenotype identification, a three-variable model was developed. This model was applied to patients in VASST (Vasopressin and Septic Shock Trial), and the identified AKI subphenotypes had a differential response to the addition of vasopressin relative to norepinephrine therapy. Identification of AKI subphenotypes could improve risk prognostication and may be useful for predictive enrichment, such that a patient's AKI subphenotype status could inform the vasopressor response.

Acute kidney injury (AKI) occurs commonly in the ICU (1) and is associated with increased morbidity and mortality (2, 3). Currently, no specific safe and effective therapeutic interventions exist for AKI other than supportive care and renal replacement therapy (RRT) (4). One reason for this may be that heterogeneity exists within the AKI patient population as currently defined, thereby hampering the identification of specific pathophysiologic pathways and therapeutic targets. The Kidney Disease: Improving Global Outcomes (KDIGO) consensus group defines AKI as an increase in serum creatinine (SCr) of ≥ 0.3 mg/dl or $>50\%$ of baseline within a 48-hour period (4). This classification system has standardized the identification of AKI. However, this system is imprecise and does not address the diversity of the underlying pathophysiology and outcomes of AKI (5).

In other heterogeneous clinical syndromes, such as acute respiratory distress syndrome (ARDS) and asthma, identification of subphenotypes has led to an improved understanding of pathogenesis and the development of precision medicine approaches (6–9). A subphenotype is a class within a population of individuals with a disease or syndrome who share clinical and physiological characteristics. We hypothesize that latent, currently unknown subgroups exist within the AKI phenotype that are biologically distinct, have different risks for adverse clinical outcomes, and respond differently to therapeutics.

In this study, we used clinical variables and circulating levels of biomarkers to identify and replicate AKI subphenotypes via a latent class analysis (LCA) approach in two independent ICU populations. We selected circulating biomarkers confined to the endothelium or inflammation/apoptosis pathways to include in the LCA modeling. We examined the associations between AKI subphenotypes and clinical outcomes, and developed a parsimonious classification model to facilitate the identification of these subphenotypes early after ICU admission. Next, we applied the AKI subphenotype classification model to patients in VASST (Vasopressin and Septic Shock Trial), and determined whether these subphenotypes responded differently to norepinephrine alone versus norepinephrine and vasopressin (10). Some of the results of this study have been previously reported in the form of an abstract (11).

Methods

Patient Population

To identify AKI subphenotypes, we performed a secondary analysis of two prospectively collected ICU data sets. The discovery group consisted of 794 patients enrolled at Harborview Medical Center (Seattle, WA) who had been admitted to an ICU with systemic inflammatory response syndrome and suspected infection (12). The replication group ($n = 425$) was assembled as part of a genome-wide association study of risk for ARDS (Table E1 in the online supplement). Sepsis was the inciting renal insult for the majority of patients in both groups. In VASST, patients with septic shock who were receiving a

minimum of 5 μg of norepinephrine per minute were randomized to receive either low-dose vasopressin (0.01–0.03 U/min) or norepinephrine (5–15 $\mu\text{g}/\text{min}$) in addition to open-label vasopressors. A random subset of patients from VASST ($n = 328$) had Ang-1 (angiopoietin-1), Ang-2, and IL-8 measured at study enrollment and received study drug infusion (13). In all cohorts, patients requiring dialysis before admission were excluded. Each cohort's local institutional review board approved the study.

In the discovery and replication groups, AKI was defined as an increase in SCr of ≥ 0.3 mg/dl or 50% from the lowest SCr value within 48 hours of study enrollment or daily urine output of <0.5 ml/kg/h within 48 hours of study enrollment. Patients with AKI were then staged based on SCr and urine output thresholds (14). In VASST, because patients were randomized early after study enrollment, a stepwise method was used to determine AKI. In patients without known chronic renal disease, the baseline SCr was back calculated using the Modification of Diet in Renal Disease (MDRD) equation assuming a baseline estimated glomerular filtration rate of 75 ml/min/1.73 m² (15). In patients with known chronic renal disease, the lowest SCr before randomization was used to estimate the baseline SCr. In all patients, urine output data obtained within the 6 hours before randomization were also used to define AKI.

Previous studies have shown that the MDRD equation may overestimate the incidence of AKI (16). In a sensitivity analysis, we also defined AKI based on a change in SCr or urine output over the first 48 hours of the study. The majority of patients were classified with AKI using either definition (78%), with the definition using the MDRD equation being more inclusive ($n = 261$) compared with the change in SCr over 48 hours ($n = 225$).

The primary outcomes were mortality after ICU admission (at 28 or 90 d). Because all populations included critically ill subjects with high rates of death, the secondary outcome of 7-day renal nonrecovery accounted for competing risk. The composite outcome of 7-day renal nonrecovery was defined as death, incident dialysis, or lack of resolution of AKI (return of SCr to within 0.3 mg/dl of baseline) by Day 7 (17). In the replication group, the

timing of incident dialysis was not available.

Statistical Analysis

Baseline clinical data and biomarker levels hypothesized to be involved in the development of AKI were considered as subphenotype-defining variables in the LCA model (18, 19). Plasma biomarkers involved in two pathways—endothelial activation/dysfunction and inflammation/apoptosis—were measured. The list of clinical variables and molecular markers were decided *a priori* based on literature supporting their role in the development in AKI (14, 19–22). All molecular markers were collected within 24 hours after AKI diagnosis (Table E2).

We applied LCA to the discovery and replication groups independently. Subphenotype classification was conducted without inclusion of clinical outcomes. We used the Vuong-Lo-Mendell-Rubin (VLMR) likelihood ratio test (tests whether n class is a better model than $n - 1$ class) as our primary test for model fit based on prior reports (23). We also considered additional criteria, including the Bayesian Information Criteria (lower values suggest model parsimony), the entropy statistic (a measure of

class separation, with optimal values greater than 0.8), class interpretability (the extent to which additional classes provided unique information), and class prevalence (preferring classes with at least 15% of the sample for improved replicability) (24).

The following analytical steps were completed: first, AKI subphenotypes were tested for associations with clinical outcomes, adjusting for Acute Physiology and Chronic Health Evaluation (APACHE) III scores and KDIGO stage within 48 hours of study enrollment. Second, Least Absolute Shrinkage and Selection Operator (LASSO) (25) was used to develop a classification model to identify subphenotype membership. Third, a Youden index, which maximizes sensitivity and specificity, was identified in the discovery group and carried forward to the replication group. Fourth, the AKI classification model was applied to VASST. Fifth, relative risk regression was used to determine risk for clinical outcomes in VASST by AKI subphenotypes, adjusting for APACHE II scores (measured in the 24 hours before randomization) and baseline norepinephrine dose before randomization. Additional details regarding the methods used in this work can be found in the online supplement.

Results

LCA: Identification of AKI Subphenotypes in the Discovery and Replication Groups

The clinical characteristics of patients with AKI in the discovery ($n = 794$) and replication ($n = 425$) groups are presented in Table 1. In the LCA models, a similar set of 29 different variables was used (Figure 1). Table E3 provides a summary of the model fits for two to four classes. In both groups, the VLMR test indicated that a two-class model was a significant improvement over a one-class model (discovery $P = 0.004$; replication $P = 0.004$). The three- and four-class models did not significantly increase the explanatory power of class identification in either group. In the two-class model, the average latent class probabilities for class designation ranged from 0.96 to 0.98 within both groups, indicating very strong probabilities for class assignment. We labeled the two-class model AKI subphenotype 1 (AKI-SP1) and AKI subphenotype 2 (AKI-SP2).

To determine whether these subphenotypes were specific to the AKI population, LCA was completed in patients without AKI from the discovery population ($n = 459$). The population without AKI had

Table 1. Clinical Characteristics of the Discovery and Replication Groups

	Discovery ($n = 794$)	Replication ($n = 425$)	VASST ($n = 256$)
Baseline demographics			
Age, yr	55 $\pm$ 16	57 $\pm$ 18	60 $\pm$ 16
Male	520 (65)	249 (59)	152 (59)
Race			
White	575 (77)	425 (100)	232 (91)
Comorbidities			
Diabetes mellitus	238 (30)	112 (27)	62 (26)
Cirrhosis	75 (9)	20 (5)	18 (7)
ICU events			
Sepsis-3	561 (71)	310 (73)	256 (100)
Vasopressors	232 (29)	237 (56)	256 (100)
Laboratory values			
Lowest sodium bicarbonate, mEq/L	20 $\pm$ 6	20 $\pm$ 5	18 $\pm$ 8
Lowest platelets, 10^9 /L	176 $\pm$ 106	158 $\pm$ 102	65 $\pm$ 106
Maximum serum creatinine, mg/dl	2.1 $\pm$ 2.2	2.4 $\pm$ 1.6	2.9 $\pm$ 1.7
Biomarker concentrations			
Ang-2/Ang-1	4 (1–15)	21 (6–77)	6 (2–11)
sTNFR-1, pg/ml	10,151 (5,926–17,079)	14,368 (8,504–23,731)	—
IL-8, pg/ml	14 (6–34)	23 (12–54)	48 (20–148)
Primary outcome			
28-d mortality	111 (14)	93 (22)	87 (34)

Definition of abbreviations: Ang-2/Ang-1 = angiotensin-2/angiotensin-1; sTNFR-1 = soluble tumor necrosis factor receptor 1; VASST = Vasopressin and Septic Shock Trial.

Continuous variables are expressed as mean $\pm$ SD or median (25th–75th percentile). Categorical variables are expressed as n (%). All variables were collected at the time of study enrollment.

Figure 1. (A and B) Heat map of the standardized values of each variable by acute kidney injury (AKI) subphenotype for the discovery (A) and replication (B) groups. The variables are sorted based on the degree of separation between AKI subphenotypes, from maximum values with AKI subphenotype 2 (AKI-SP2) at the top to maximum values for AKI-SP1 at the bottom. Variables were standardized by scaling all means to zero, with SD = 1. A variable of 1+ for the standardized variable signifies that the mean value for a given AKI subphenotype was an entire SD higher than the mean value for the population as a whole in that group (discovery or replication).

lower severity-of-illness scores, lower SCr values (average 0.9 mg/dl), and lower plasma biomarker concentrations than the AKI population in the discovery group (Table E4). Even though the same set of variables was used in the modeling, LCA did not identify a multiclass model that provided a better fit than a one-class model in this population without AKI (Table E5). We then addressed the possibility that collinearity of variables was influencing class separation. After exclusion of collinear variables based on a conservative Pearson's pairwise correlation

threshold > 0.5, a two-class model continued to best fit the data. Less than 2% of patients changed class assignment (Tables S6).

Clinical and Biological Characteristics of AKI Subphenotypes

In both groups, multiple clinical and biological variables differentiated AKI-SP1 from AKI-SP2. Figure 1 provides a heat map of the variables included in the LCA modeling, sorted by the degree of separation between the subphenotypes. Compared with AKI-SP1, AKI-SP2 was

characterized by worse renal function, higher rates of sepsis, vasopressor use, and ARDS (Table 2). AKI subphenotypes did not differ by age, sex, or body mass index, but markers of endothelial activation (plasma Ang-1, Ang-2, and platelet concentrations) and inflammation (plasma sTNFR-1 [soluble tumor necrosis factor receptor 1], sodium bicarbonate, IL-6, and IL-8) were markedly different between AKI-SP1 and AKI-SP2. Overall, the characteristics of AKI-SP1 and AKI-SP2 were consistent between the discovery and replication groups.

Table 2. Patient Characteristics and Outcomes Based on AKI Subphenotypes in the Discovery and Replication Populations

	Discovery			Replication		
	AKI-SP1	AKI-SP2	P Value	AKI-SP1	AKI-SP2	P Value
Subjects	462	332	—	268	157	—
Age, yr	55 ± 16	55 ± 16	0.80	57 ± 18	57 ± 18	0.95
Male	296 (65)	224 (66)	0.62	164 (61)	104 (39)	0.15
Race						
White	331 (76)	244 (78)	0.57	268 (100)	157 (100)	—
Comorbidities						
Diabetes mellitus	129 (28)	109 (32)	0.21	72 (27)	40 (26)	0.73
Cirrhosis	8 (2)	67 (20)	<0.01	6 (2)	14 (9)	<0.01
Chronic kidney disease	31 (7)	70 (21)	<0.01	—	—	—
ICU events*						
APACHE III scores	47 ± 21	72 ± 29	<0.01	74 ± 24	111 ± 26	<0.01
SOFA Day 1 scores	3 ± 2.3	7 ± 3.0	<0.01	8 ± 2	11 ± 3	<0.01
Sepsis-3	257 (46)	304 (54)	<0.01	178 (66)	132 (84)	<0.01
Acute respiratory distress syndrome	43 (9)	74 (22)	<0.01	107 (40)	103 (66)	<0.01
Vasopressors	66 (14)	166 (49)	<0.01	113 (42)	124 (79)	<0.01
Mechanical ventilation	286 (63)	280 (83)	<0.01	198 (74)	134 (85)	<0.01
KDIGO class 2 + 3	84 (19)	121 (36)	<0.01	48 (18)	45 (29)	<0.01
24-h urine output, ml	1,555 (910–2,565)	1,225 (486–2,089)	<0.01	1,680 (1,140–2,665)	1,199 (563–2,050)	<0.01
ICU laboratory values*						
Maximum white blood cell count, 10 ⁹ /L	14 ± 7	16 ± 10	<0.01	16 ± 8	17 ± 13	<0.01
Low hematocrit, %	30 ± 6	31 ± 6	0.77	30 ± 6	31 ± 6	0.77
Low sodium, mEq/L	137 ± 6	135 ± 5	<0.01	137 ± 6	135 ± 5	<0.01
Low albumin, g/dl	2.4 ± 0.6	2.2 ± 0.7	<0.01	2.4 ± 0.6	2.2 ± 0.7	<0.01
Low platelets, 10 ⁹ /L	184 ± 101	85 ± 75	<0.01	184 ± 101	85 ± 75	<0.01
Low sodium bicarbonate, mEq/L	22 ± 5	17 ± 5	<0.01	22 ± 5	17 ± 5	<0.01
Maximum serum creatinine, mg/dl	1.4 ± 0.8	3.1 ± 2.9	<0.01	1.9 ± 1.4	3.1 ± 1.7	<0.01
Biomarker concentrations						
Ang-2/Ang-1 ratio	1.4 (0.7–3.2)	18.1 (8.2–53.9)	<0.01	9.4 (3.3–25.5)	87.1 (35.7–266.1)	<0.01
sTNFR-1, pg/ml	6,798 (4,701–10,108)	18,772 (12,663–30,889)	<0.01	10,581 (6,828–15,742)	25,815 (16,084–36,211)	<0.01
IL-8, pg/ml	10 (5–21)	22 (12–55)	<0.01	16 (10–26)	60 (28–149)	<0.01

	Discovery				Replication			
	AKI-SP1	AKI-SP2	RR (95% CI) [†]	P Value	AKI-SP1	AKI-SP2	RR (95% CI) [†]	P Value
Clinical outcomes								
7-d renal nonrecovery	16 (3)	78 (23)	4.4 (2.5–7.9)	<0.001	66 (25)	72 (46)	1.6 (1.1–2.2)	0.006
28-d mortality	28 (6)	83 (25)	2.5 (1.6–4.1)	<0.001	36 (13)	57 (36)	2.2 (1.3–3.5)	0.002

Definition of abbreviations: AKI-SP1/2 = acute kidney injury subphenotype 1/2; Ang-2/Ang-1 = angiotensin-2/angiotensin-1; APACHE III = Acute Physiology and Chronic Health Evaluation III; CI = confidence interval; KDIGO = Kidney Disease: Improving Global Outcomes; RR = relative risk; SOFA = sequential organ failure assessment; sTNFR-1 = soluble tumor necrosis factor receptor 1.

*All ICU events and ICU laboratory values are the maximum or minimum value within 48 hours of study enrollment. Data are shown as mean ± SD, *n* (%), or median (interquartile range), as appropriate.

[†]Relative risk estimates for AKI-SP2 compared with AKI-SP1 were adjusted for APACHE III scores and KDIGO stage of AKI.

Association of AKI Subphenotypes with Clinical Outcomes

The risk of 28-day mortality was greater with AKI-SP2 relative to AKI-SP1, adjusting for APACHE III scores and KDIGO stages of AKI on study enrollment (discovery: adjusted relative risk [aRR], 2.5 [95% CI, 1.6–4.1]; replication: aRR, 2.2 [95% CI,

1.3–3.5]) (Table 2). Similar results were obtained after adjusting for sequential organ failure assessment (SOFA) scores and KDIGO stages of AKI (Table E9). The adjusted risk of 7-day renal nonrecovery was also significantly greater with AKI-SP2 than with AKI-SP1 (discovery: aRR, 4.4 [2.5–7.9]; replication:

aRR, 1.6 [1.1–2.2]) (Table 2). Furthermore, the AKI subphenotypes better separated risk for renal specific outcomes than the KDIGO stage of AKI (Table E10).

Classification of AKI Subphenotypes

Next, LASSO regression was used to identify a parsimonious set of variables that could

Table 3. Classification Model Performance for AKI Subphenotype Membership in Both Groups

AKI Subphenotype Classification Model	Discovery (Split 60/40)		Replication: External Validation (n = 425)
	Derivation (n = 476)	Internal Validation (n = 318)	
Ang-2/Ang-1 + sTNFR-1, C-statistic (95% CI)*	0.98 (0.97–0.99)	0.97 (0.96–0.99)	0.93 (0.91–0.95)
Sensitivity	0.90	0.90	0.86
Specificity	0.95	0.95	0.83
Positive predictive value	0.92	0.94	0.75
Negative predictive value	0.93	0.92	0.91
Ang-2/Ang-1 + IL-8, C-statistic (95% CI)†	0.95 (0.94–0.97)	0.95 (0.92–0.97)	0.92 (0.89–0.94)
Sensitivity	0.88	0.90	0.88
Specificity	0.87	0.84	0.76
Positive predictive value	0.82	0.82	0.68
Negative predictive value	0.92	0.91	0.91

Definition of abbreviations: AKI = acute kidney injury; Ang-2/Ang-1 = angiopoietin-2/angiopoietin-1; CI = confidence interval; sTNFR-1 = soluble tumor necrosis factor receptor 1.

*Least Absolute Shrinkage and Selection Operator regression was used for classification model development. The “gold standard” for AKI subphenotype was assignment by latent class analysis. Youden’s index threshold, which maximizes sensitivity and specificity, was chosen in the derivation group. This threshold was then carried forward to the internal and external validation groups.

†The modified AKI classification model was developed substituting IL-8 for sTNFR-1.

discriminate AKI subphenotypes. The “gold standard” for AKI subphenotype was assignment by LCA. The ratio of plasma Ang-2/Ang-1 and sTNFR-1 concentrations resulted in a classification model with a high C-statistic, ≥ 0.93 (Table 3). The inclusion of any additional variable to the classification model did not significantly improve the C-statistic ($P = 0.11$). The Youden’s index of the three-variable classification model derived in the discovery group accurately predicted AKI subphenotypes in the replication group (positive predictive value 0.72 and negative predictive value 0.93). The classification model was well calibrated (Figure E2). The biomarkers included in the three-variable classification model improved the prediction of clinical outcomes, potentially demonstrating the importance of Ang-2/Ang-1 and sTNFR-1 in risk prognostication in AKI (Tables E11 and E12).

AKI subphenotypes classified by the three-variable classification model had similar risks of death and poor renal outcomes compared with AKI subphenotypes classified by LCA (Table E13). A modified version of the classification model was developed, replacing IL-8 with sTNFR-1, as sTNFR-1 was not available in VASST. The modified classification model discriminated AKI subphenotypes well (C-statistic ≥ 0.92 in the populations) and was not significantly different from the LASSO-developed model.

AKI Subphenotype Response to Vasopressin

In VASST, a subset of 328 patients had IL-8, Ang-1, and Ang-2 measured, and among these patients, 271 had AKI (Table 1). Characteristics and related outcomes in the subset were representative of VASST overall. The mean age was 60 years, 23% had diabetes mellitus, 12% had chronic kidney disease, and the median APACHE II score was 27. The adjusted risk of 28-day or 90-day mortality did not differ significantly by treatment group, consistent with the original study results ($P = 0.45$ and $P = 0.14$, respectively). A three-variable classification model (Ang-1, Ang-2, and IL-8) classified 113 patients as AKI-SP1 and 148 as AKI-SP2 (Table 4). In AKI-SP1, early addition of vasopressin compared with norepinephrine alone was associated with improved 90-day mortality (27% vs. 46%, respectively; $P = 0.02$), but in AKI-SP2, vasopressin showed no significant treatment difference (45% vs. 49%, respectively; $P = 0.99$) (Table 4). A test for interaction between AKI subphenotypes and vasopressin for 90-day mortality resulted in a P value of 0.05. Vasopressin was also associated with a differential risk of 28-day mortality, although the test for interaction did not achieve statistical significance ($P = 0.11$).

We performed a sensitivity analysis to determine whether differences in severity of

illness might be driving the differential response between AKI subphenotypes. There was no interaction between APACHE II score (as a continuous variable or when dichotomized about the median) and vasopressin for 90-day mortality ($P = 0.75$ and $P = 0.77$, respectively). We also tested whether expanding the window of time for defining AKI to within 48 hours of study enrollment would change our results. We continued to find that vasopressin was beneficial among patients classified as AKI-SP1, but not in those classified as AKI-SP2 (Table E14).

Discussion

Current classification systems for AKI rely exclusively on SCr and urine output, and the classes defined by these systems show heterogeneity in biology (26), recovery patterns (27), and subsequent outcomes (28). In contrast, the application of LCA to a panel of 29 clinical and biomarker variables in two critically ill AKI populations identified two novel AKI subphenotypes with differing risks for clinical outcomes. In addition, the strength of association for poor renal outcomes was greater with AKI subphenotypes than the KDIGO stage of AKI within 48 hours after ICU admission. A parsimonious classification model that included markers of endothelial dysfunction (Ang-2/Ang-1) and

Table 4. Clinical Characteristics Based on AKI Subphenotypes in VASST

	AKI-SP1	AKI-SP2	P Value
Subjects	113	143	
Baseline demographics			
Age, yr	61 ± 16	59 ± 16	0.29
Male	72 (64)	80 (56)	0.21
Race			
White	107 (95)	125 (87)	0.13
Comorbidities			
Diabetes mellitus	27 (27)	35 (26)	0.92
Chronic kidney disease	5 (5)	21 (15)	0.01
ICU events*			
Baseline norepinephrine, µg/min	9 ± 13	14 ± 22	0.01
APACHE II	25 ± 7	30 ± 7	<0.01
Low platelets, 10 ⁹ /L	232 ± 130	133 ± 94	<0.01
Low bicarbonate, mEq/L	20 ± 9	18 ± 8	0.02
Urine output, ml/kg/h	1.3 ± 1.3	1.3 ± 2.6	0.45
Laboratory values			
Maximum serum creatinine, mg/dl	2.6 ± 1.9	3.2 ± 1.5	<0.01
Biomarkers			
Ang-2/Ang-1	2 (0.7–2.8)	10 (7–16)	<0.01
IL-8, pg/ml	23 (13–47)	106 (49–345)	<0.01

	AKI-SP1				AKI-SP2			
	Norepinephrine	Vasopressin	RR (95% CI) [†]	P Value	Norepinephrine	Vasopressin	RR (95% CI) [†]	P Value
Clinical outcomes								
7-d renal nonrecovery	24 (46)	23 (38)	0.80 (0.51–1.25)	0.32	44 (63)	44 (56)	0.99 (0.76–1.30)	0.96
28-d mortality	16 (31)	11 (18)	0.53 (0.30–0.94)	0.03	30 (43)	31 (40)	1.03 (0.68–1.55)	0.88
90-d mortality	24 (46)	16 (27)	0.54 (0.32–0.92)	0.02	34 (49)	35 (45)	0.99 (0.70–1.42)	0.99

Definition of abbreviations: AKI-SP1/2 = acute kidney injury subphenotype 1/2; Ang-2/Ang-1 = angiotensin-2/angiotensin-1; APACHE = Acute Physiology and Chronic Health Evaluation; CI = confidence interval; RR = relative risk; VASST = Vasopressin and Septic Shock Trial.

P value for interaction = 0.11 for 28-d mortality and 0.05 for 90-d mortality.

*All ICU events and ICU laboratory values are the maximum or minimum value within 48 hours of study enrollment. Data are shown as mean ± SD, *n* (%), or median (interquartile range), as appropriate.

[†]Relative risk estimates for vasopressin compared with norepinephrine were adjusted for APACHE II scores.

inflammation (sTNFR-1 or IL-8) accurately identified AKI subphenotypes, highlighting the importance of these biological processes. Additionally, AKI subphenotypes showed a differential response to vasopressin therapy with AKI-SP1 or the less “severe” form of AKI, showing a benefit with vasopressin therapy. In accordance with precision medicine concepts, the identification of AKI subphenotypes linked to underlying biological processes may begin to parse the heterogeneity in the AKI phenotype and identify treatment-responsive AKI subtypes.

In clinical AKI, it has been particularly problematic to identify clinical subgroups with biologically distinct signatures. For example, clinicians have historically separated AKI into prerenal and acute tubular necrosis. However, multiple studies

have shown poor reliability of biomarkers or urine studies in differentiating these two groups (29, 30). In contrast, we have identified a three-variable classification model that accurately identifies AKI subphenotypes. This molecular classification model could allow the identification of patients most likely to benefit from drugs targeting pathways of endothelial dysfunction or inflammation in clinical trials. Finally, this simple model could be useful as a decision support tool to inform triaging and management decisions early in the course of critical illness.

Although LASSO regression identified a simplified three-variable model to identify AKI subphenotypes, future work is necessary to evaluate the biological role of these plasma biomarkers (Ang-2/Ang-1 and sTNFR-1) in the development of AKI

subphenotypes. However, multiple prior reports have implicated biomarkers of endothelial function and inflammation in the pathophysiology of AKI (31–33). Ang-1 and Ang-2 are vascular endothelial growth factors that both bind to the endothelial tyrosine kinase receptor Tie-2 but have context-dependent activities (33). Ang-1 is an agonist for Tie-2 and plays a protective role by stabilizing the endothelium and preventing microcirculatory capillary leakage, a hallmark of AKI (34). In contrast, Ang-2 typically acts as an antagonist to the Tie-2 receptor and promotes endothelial dysfunction (35) and inflammation (18). Animal studies have shown that administration of recombinant Ang-1 (36, 37) or activation of Tie-2 (38) decreases endothelial leak and protects against AKI. In the kidney, sTNFR-1

is expressed in the glomerular endothelium and plays a causative role in the development of endothelial cell dysfunction, inflammation, and AKI (39). Taken together, these findings provide biological plausibility that markers of endothelial dysfunction and inflammation are linked to the development of specific AKI subphenotypes.

In critical illness, vasopressors play an essential role in cardiovascular management and organ perfusion. In the kidneys, vasopressin binds to AVP-1 receptors of glomerular efferent arterioles, leads to arteriolar vasoconstriction, and in turn increases glomerular filtration (40). In contrast, norepinephrine binds to α -1 receptors of glomerular afferent arterioles and decreases glomerular perfusion pressure and filtration. Previous small studies have shown that vasopressin therapy spares alternative catecholamine use, and compared with norepinephrine is associated with significantly increased urine output and creatinine clearance (41). Thus, our findings build on this foundation of knowledge and encourage the development of bedside assays to identify AKI subphenotypes and test our findings in future clinical trials.

Our findings are potentially consistent with the results of the VANISH (Effect of Early Vasopressin versus Norepinephrine on Kidney Failure in Patients with Septic Shock) study (42). Although this trial did not show a benefit in mortality, the CIs for the risk estimates were quite wide, suggesting heterogeneity in treatment responses within the studied population. In addition, the secondary outcome of incident dialysis was significantly better in the vasopressin group than in the norepinephrine group (25% for vasopressin vs. 35% for norepinephrine). Thus, reanalysis of VASST may have identified a subpopulation that responds to vasopressin therapy, and this treatment signal may have been previously hidden due to clinical heterogeneity in the AKI population.

It is important to note that the early addition of vasopressin did not affect the risk of 7-day renal nonrecovery in the AKI subphenotypes, whereas the risk of 28- and 90-day mortality differed significantly between the two groups. Potential explanations for this discrepancy in results could include competing risks, i.e., the subjects could have experienced one or more events (death or discharge) that

competed with the outcome of interest, renal recovery. Alternatively, in a population of patients with septic shock and AKI, 7 days may be too proximal a time point for renal recovery. Future studies with long-term outpatient assessments of kidney function in survivors of AKI may identify a difference in renal recovery between AKI subphenotypes.

Our study has several strengths. First, LCA identified AKI subphenotypes with similar characteristics in two independent ICU populations, and the association of these AKI subphenotypes with renal and other outcomes persisted even after adjusting for measures of ICU severity of illness (APACHE III or SOFA scores). Second, the only variables used in our LCA models were ascertained very early in the course of critical illness and AKI. Thus, the AKI subphenotypes we have identified are reflective of the state of patients at a time when critical prognostic and therapeutic decisions are being made. Third, to improve the clinical utility of our findings, we developed a simplified three-variable classification model that accurately identified AKI subphenotypes. The three-variable classification model would allow the prospective identification of AKI subphenotypes during clinical care. Fourth, AKI subphenotypes responded differently to vasopressor therapy in patients with septic shock. Future studies will need to clarify whether prognostic enrichment for the AKI subphenotypes in clinical trials will overcome the noise within the heterogeneous AKI phenotype and potentially aid in the identification of novel treatments for AKI.

This study has several important limitations. First, urinary biomarkers of tubular injury or cell cycle arrest were not used in the LCA modeling. Urine was not collected in any of the AKI populations. However, the use of circulating biomarkers of endothelial function and inflammation may allow the identification of novel AKI subphenotypes that are not specifically defined by the presence or absence of kidney epithelial injury. Future research to characterize the urinary biomarker profile of AKI subphenotypes is warranted, and we advocate the collection of urine biosamples in all future critical-illness cohort studies. Second, we do not know the extent to which changes in renal clearance influence biomarker levels, and thus we cannot exclude the possibility that a portion of the

associations observed between biomarker levels and renal outcomes is due to the biomarker acting as a surrogate for SCr. Previous studies have shown that plasma and urinary levels of Ang-2 are both increased in chronic kidney disease (43, 44), and neither plasma TNF- α , the natural ligand for TNFR-1, nor Ang-2 are cleared by dialysis, suggesting that decreased glomerular filtration is unlikely to be the sole explanation for differences in biomarker levels (45). Third, in the analyses in VASST, we used the MDRD equation to identify patients with AKI who did not have chronic renal disease. Although previous studies have shown that this criterion may be overly sensitive (16), the median SCr before randomization in VASST was greater than 2 mg/dL, suggesting that we identified an AKI population with moderate to severe renal injury. As a sensitivity analysis, we also adjudicated AKI in VASST 48 hours after randomization, and found similar response between vasopressin and AKI subphenotypes. Thus, we have taken a rigorous approach to identify patients with AKI using multiple different definitions. Fourth, the differences in outcomes between the AKI subphenotypes may be related to severity of illness. However, all analyses adjusted for severity of illness (APACHE or SOFA) and the differences in vasopressin response in VASST were not seen if APACHE II was used to stratify patients. In addition, the group that responded to vasopressin therapy was AKI-SP1, not the population with more severe illness, i.e., AKI-SP2.

Conclusions

We have defined two distinct AKI subphenotypes that exhibit differences with regard to risk for adverse clinical outcomes, underlying pathophysiology, and response to vasopressin therapy. In the future, AKI subphenotypes may allow for improved biological characterization of AKI in the critically ill, and may facilitate prognostic and predictive enrichment for enrollment in clinical trials. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

Acknowledgment: The authors thank Gail Rona and Shirley Whitkanak, research coordinators involved in the recruitment of subjects.

References

- Joannidis M, Druml W, Forni LG, Groeneveld ABJ, Honore PM, Hoste E, et al. Prevention of acute kidney injury and protection of renal function in the intensive care unit: update 2017: expert opinion of the Working Group on Prevention, AKI section, European Society of Intensive Care Medicine. *Intensive Care Med* 2017;43:730–749.
- Chertow GM, Burdick E, Honour M, Bonventre JV, Bates DW. Acute kidney injury, mortality, length of stay, and costs in hospitalized patients. *J Am Soc Nephrol* 2005;16:3365–3370.
- Nisula S, Kaukonen K-M, Vaara ST, Korhonen A-M, Poukkanen M, Karlsson S, et al.; FINNAKI Study Group. Incidence, risk factors and 90-day mortality of patients with acute kidney injury in Finnish intensive care units: the FINNAKI study. *Intensive Care Med* 2013;39:420–428.
- Davison SN, Levin A, Moss AH, Jha V, Brown EA, Brennan F, et al.; Kidney Disease: Improving Global Outcomes. Executive summary of the KDIGO Controversies Conference on Supportive Care in Chronic Kidney Disease: developing a roadmap to improving quality care. *Kidney Int* 2015;88:447–459.
- Barasch J, Zager R, Bonventre JV. Acute kidney injury: a problem of definition. *Lancet* 2017;389:779–781.
- Calfee CS, Delucchi K, Parsons PE, Thompson BT, Ware LB, Matthay MA; NHLBI ARDS Network. Subphenotypes in acute respiratory distress syndrome: latent class analysis of data from two randomised controlled trials. *Lancet Respir Med* 2014;2:611–620.
- Famous KR, Delucchi K, Ware LB, Kangelaris KN, Liu KD, Thompson BT, et al.; ARDS Network. Acute respiratory distress syndrome subphenotypes respond differently to randomized fluid management strategy. *Am J Respir Crit Care Med* 2017;195:331–338.
- Moore WC, Meyers DA, Wenzel SE, Teague WG, Li H, Li X, et al.; National Heart, Lung, and Blood Institute's Severe Asthma Research Program. Identification of asthma phenotypes using cluster analysis in the Severe Asthma Research Program. *Am J Respir Crit Care Med* 2010;181:315–323.
- Corren J, Lemanske RF, Hanania NA, Korenblat PE, Parsey MV, Arron JR, et al. Lebelrikizumab treatment in adults with asthma. *N Engl J Med* 2011;365:1088–1098.
- Russell JA, Walley KR, Singer J, Gordon AC, Hébert PC, Cooper DJ, et al.; VASST Investigators. Vasopressin versus norepinephrine infusion in patients with septic shock. *N Engl J Med* 2008;358:877–887.
- Bhatraju PK, Robinson-Cohen C, Himmelfarb J, Wurfel MM. Latent class analysis identifies AKI sub-phenotypes with differing biomarker profiles of endothelial dysfunction and relation to risk of death and need for renal replacement therapy [abstract]. *J Am Soc Nephrol Abstr Suppl* 27:244A.
- Glavan BJ, Holden TD, Goss CH, Black RA, Neff MJ, Nathens AB, et al.; ARDSnet Investigators. Genetic variation in the FAS gene and associations with acute lung injury. *Am J Respir Crit Care Med* 2011;183:356–363.
- Russell JA, Lee T, Singer J, Boyd JH, Walley KR; Vasopressin and Septic Shock Trial (VASST) Group. The septic shock 3.0 definition and trials: a vasopressin and septic shock trial experience. *Crit Care Med* 2017;45:940–948.
- Kellum JA, Lameire N; KDIGO AKI Guideline Work Group. Diagnosis, evaluation, and management of acute kidney injury: a KDIGO summary (part 1). *Crit Care* 2013;17:204.
- Thomas ME, Blaine C, Dawney A, Devonald MAJ, Ftouh S, Laing C, et al. The definition of acute kidney injury and its use in practice. *Kidney Int* 2015;87:62–73.
- Bouchard J, Macedo E, Soroko S, Chertow GM, Himmelfarb J, Ikizler TA, et al.; Program to Improve Care in Acute Renal Disease. Comparison of methods for estimating glomerular filtration rate in critically ill patients with acute kidney injury. *Nephrol Dial Transplant* 2010;25:102–107.
- Forni LG, Darmon M, Ostermann M, Oudemans-van Straaten HM, Pettilä V, Prowle JR, et al. Renal recovery after acute kidney injury. *Intensive Care Med* 2017;43:855–866.
- Woolf AS, Gnudi L, Long DA. Roles of angiotensins in kidney development and disease. *J Am Soc Nephrol* 2009;20:239–244.
- Liu KD, Glidden DV, Eisner MD, Parsons PE, Ware LB, Wheeler A, et al.; National Heart, Lung, and Blood Institute ARDS Network Clinical Trials Group. Predictive and pathogenetic value of plasma biomarkers for acute kidney injury in patients with acute lung injury. *Crit Care Med* 2007;35:2755–2761.
- Robinson-Cohen C, Katz R, Price BL, Harju-Baker S, Mikacenic C, Himmelfarb J, et al. Association of markers of endothelial dysregulation Ang1 and Ang2 with acute kidney injury in critically ill patients. *Crit Care Med* 2007;35:2755–2761.
- Bagshaw SM, George C, Gibney RTN, Bellomo R. A multi-center evaluation of early acute kidney injury in critically ill trauma patients. *Ren Fail* 2008;30:581–589.
- Hoste EAJ, Bagshaw SM, Bellomo R, Cely CM, Colman R, Cruz DN, et al. Epidemiology of acute kidney injury in critically ill patients: the multinational AKI-EPI study. *Intensive Care Med* 2015;41:1411–1423.
- Nylund KL, Asparouhov T, Muthén BO. Deciding on the number of classes in latent class analysis and growth mixture modeling: a Monte Carlo simulation study. *Struct Equ Modeling* 2007;14:535–569.
- Nguyen HQ, Herting JR, Pike KC, Gharib SA, Matute-Bello G, Borson S, et al. Symptom profiles and inflammatory markers in moderate to severe COPD. *BMC Pulm Med* 2016;16:173.
- Tibshirani R. Regression shrinkage and selection via the lasso: a retrospective. *J R Stat Soc Series B Stat Methodol* 2011;73:273–282.
- Xu K, Rosenstiel P, Paragas N, Hinze C, Gao X, Shen TH, et al. Unique transcriptional programs identify subtypes of AKI. *J Am Soc Nephrol* 2017;28:1729–1740.
- Kellum JA, Sileanu FE, Bihorac A, Hoste EA, Chawla LS. Recovery after acute kidney injury. *Am J Respir Crit Care Med* 2017;195:784–791.
- Bhatraju PK, Mukherjee P, Robinson-Cohen C, O'Keefe GE, Frank AJ, Christie JD, et al. Acute kidney injury subphenotypes based on creatinine trajectory identifies patients at increased risk of death. *Crit Care* 2016;20:372.
- Bellomo R, Bagshaw S, Langenberg C, Ronco C. Pre-renal azotemia: a flawed paradigm in critically ill septic patients? *Contrib Nephrol* 2007;156:1–9.
- Uchino S. The meaning of transient azotemia. *Contrib Nephrol* 2010;165:337–344.
- Jongman RM, van Klarenbosch J, Molema G, Zijlstra JG, de Vries AJ, van Meurs M. Angiotensin/Tie2 dysbalance is associated with acute kidney injury after cardiac surgery assisted by cardiopulmonary bypass. *PLoS One* 2015;10:e0136205.
- Kümpers P, Hafer C, David S, Hecker H, Lukasz A, Fliser D, et al. Angiotensin-2 in patients requiring renal replacement therapy in the ICU: relation to acute kidney injury, multiple organ dysfunction syndrome and outcome. *Intensive Care Med* 2010;36:462–470.
- Ziegler T, Horstkotte J, Schwab C, Pfetsch V, Weinmann K, Dietzel S, et al. Angiotensin 2 mediates microvascular and hemodynamic alterations in sepsis. *J Clin Invest* 2013;123:3436–3445.
- Ricciuto DR, dos Santos CC, Hawkes M, Tolti LJ, Conroy AL, Rajwans N, et al. Angiotensin-1 and angiotensin-2 as clinically informative prognostic biomarkers of morbidity and mortality in severe sepsis. *Crit Care Med* 2011;39:702–710.
- Parikh SM, Mammoto T, Schultz A, Yuan H-T, Christiani D, Karumanchi SA, et al. Excess circulating angiotensin-2 may contribute to pulmonary vascular leak in sepsis in humans. *PLoS Med* 2006;3:e46.
- David S, Park J-K, Meurs Mv, Zijlstra JG, Koenecke C, Schrimpf C, et al. Acute administration of recombinant Angiotensin-1 ameliorates multiple-organ dysfunction syndrome and improves survival in murine sepsis. *Cytokine* 2011;55:251–259.
- Kim DH, Jung YJ, Lee AS, Lee S, Kang KP, Lee TH, et al. COMP-angiotensin-1 decreases lipopolysaccharide-induced acute kidney injury. *Kidney Int* 2009;76:1180–1191.
- Han S, Lee S-J, Kim KE, Lee HS, Oh N, Park I, et al. Amelioration of sepsis by TIE2 activation-induced vascular protection. *Sci Transl Med* 2016;8:335ra55.
- Fiedler U, Reiss Y, Scharpfenecker M, Grunow V, Koidl S, Thurston G, et al. Angiotensin-2 sensitizes endothelial cells to TNF-alpha and has a crucial role in the induction of inflammation. *Nat Med* 2006;12:235–239.

40. Edwards RM, Trizna W, Kinter LB. Renal microvascular effects of vasopressin and vasopressin antagonists. *Am J Physiol* 1989;256: F274–F278.
41. Patel BM, Chittock DR, Russell JA, Walley KR. Beneficial effects of short-term vasopressin infusion during severe septic shock. *Anesthesiology* 2002;96:576–582.
42. Gordon AC, Mason AJ, Thirunavukkarasu N, Perkins GD, Cecconi M, Cepkova M, *et al.*; VANISH Investigators. Effect of early vasopressin vs norepinephrine on kidney failure in patients with septic shock: the VANISH randomized clinical trial. *JAMA* 2016;316: 509–518.
43. Chen S, Li H, Zhang C, Li Z, Wang Q, Guo J, *et al.* Urinary angiotensin-2 is associated with albuminuria in patients with type 2 diabetes mellitus. *Int J Endocrinol* 2015;2015:163120.
44. Idasiak-Piechocka I, Oko A, Pawliczak E, Kaczmarek E, Czekalski S. Urinary excretion of soluble tumour necrosis factor receptor 1 as a marker of increased risk of progressive kidney function deterioration in patients with primary chronic glomerulonephritis. *Nephrol Dial Transplant* 2010;25:3948–3956.
45. Tarakçioğlu M, Erbağcı AB, Usalan C, Deveci R, Kocabaş R. Acute effect of hemodialysis on serum levels of the proinflammatory cytokines. *Mediators Inflamm* 2003;12:15–19.