ORIGINAL CLINICAL REPORT

OPEN

Serial Urinary C-C Motif Chemokine Ligand 14 and Risk of Persistent Severe Acute Kidney Injury

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OBJECTIVES: To assess the added prognostic value of serial monitoring of urinary C-C motif chemokine ligand 14 (uCCL14) over that of single measurements, which have been shown to be prognostic for development of persistent severe acute kidney injury (AKI) in critically ill patients.

DESIGN: Retrospective observational study.

SETTING: Data derived from two multinational ICU studies (Ruby and Sapphire).

PATIENTS: Critically ill patients with early stage 2–3 AKI.

INTERVENTIONS: None.

MEASUREMENTS AND MAIN RESULTS: We analyzed three consecutive uCCL14 measurements at 12-hour intervals after diagnosis of stage 2–3 AKI by Kidney Disease Improving Global Outcomes criteria. Primary outcome was persistent severe AKI, defined as 72 consecutive hours of stage 3 AKI, death, or receipt of dialysis prior to 72 hours. uCCL14 was measured using the NEPHROCLEAR uCCL14 Test on the Astute 140 Meter (Astute Medical, San Diego, CA). Based on predefined, validated cutoffs, we categorized uCCL14 as: low (≤ 1.3 ng/mL), medium (> 1.3 to ≤ 13 ng/mL), or high (> 13 ng/mL). Seventy-five of 417 patients with three consecutive uCCL14 measurements developed persistent severe AKI. Initial uCCL14 category strongly correlated with primary endpoint and, in most cases (66%), uCCL14 category was unchanged over the first 24 hours. Compared with no change and accounting for baseline category, decrease in category was associated with decreased odds of persistent severe AKI (odds ratio [OR], 0.20; 95% CI, 0.08–0.45; $p < 0.001$) and an increase in category with increased odds (OR, 4.04; 95% CI, 1.75–9.46; $p = 0.001$).

CONCLUSIONS: In one-third of patients with moderate to severe AKI uCCL14 risk category altered over three serial measurements and such changes were associated with altered risk for persistent severe AKI. Serial CCL-14 measurement may detect progression or resolution of underlying kidney pathology and help refine AKI prognosis.

KEY WORDS: acute kidney injury; biomarkers; C-C motif chemokine ligand 14; critical illness; prognosis

Acute kidney injury (AKI) affects more than one in four hospitalized patients and one in two admitted to intensive care (1). In the short term, AKI is associated with increased risk of death, length, and cost of hospitalization and, in survivors, greater longer-term morbidity and mortality, often associated with the development of chronic kidney disease (CKD) (2–5). In particular, persistent severe AKI (PS-AKI), defined as Kidney Disease Improving Global Outcomes (6) (KDIGO) AKI stage 3, lasting 3 or more days, has been strongly associated with adverse outcomes across a range of clinical settings (7–9). However, predicting the clinical course of AKI is difficult, in part

DOI: 10.1097/CCE.0000000000000870

KEY POINTS

Question: Does serial measurement of urinary C-C motif chemokine ligand 14 (uCCL14) provide complementary prognostic information over a single measurement early after the diagnosis of stage 2–3 acute kidney injury (AKI) complicating critical illness.

Findings: In one-third of patients with moderate to severe AKI uCCL14 risk category altered over three serial measurements and such changes were associated with altered risk for persistent severe AKI.

Meaning: Serial C-C motif chemokine ligand 14 measurement may reveal dynamic pathology and help refine prognosis.

because changes in serum creatinine may significantly lag changes in underlying glomerular filtration rate while urine output may be well preserved even in the face of significant tubular injury. Thus, a patient with newly detected stage 2 AKI could already be recovering, could have an intermediate level of kidney damage which may respond to targeted intervention or may already have severe parenchymal damage that will progress toward need for renal replacement therapy (RRT). Accordingly, better tools for identifying those at highest risk for PS-AKI are required to enable predictive enrichment of populations for AKI-targeted intervention.

Previously the Ruby study (10) described urinary C-C motif chemokine ligand 14 (uCCL14) as a novel predictor of future PS-AKI, in patients shortly after first diagnosis of stage 2–3 AKI. The ability of uCCL14 to forecast PS-AKI has subsequently been verified in two independent cohorts (11, 12). More recently in a preplanned secondary analysis, two cutoff values of uCCL14 have been described identifying groups at low, moderate, and higher risk of PS-AKI (13). However, previous analyses have been based only on the initial values of uCCL14, close to first diagnosis of moderate to severe AKI and the relationship of serial measures of uCCL14 to the development of PS-AKI have not been described. We hypothesized that as a biomarker of underlying kidney inflammation during tissue injury and repair, serial measurement of uCCL14 could be informative about clinical course of AKI. Accordingly, in a preplanned analysis of two observational studies, we sought to characterize changes in categorized uCCL14 values over time and their association with clinical outcome.

METHODS

Research Background

We conducted a secondary analysis of serial measurement of uCCL14 early after development of moderate to severe AKI in a mixed population of critically ill adults. The analytic cohort is composed of pooled patients from the previously reported Ruby and Sapphire studies (10, 14). The Ruby study (Identification and Validation of Biomarkers of Acute Kidney Injury Recovery) and the Sapphire study (Evaluation of Novel Biomarkers From Acutely Ill Patients at Risk for Acute Kidney Injury) were approved by the Western Institutional Review Board (Puyallup, WA). Sapphire (Approval Number: 20101176; Date: August 27, 2010) and Ruby (Approval Number: 20130523; Date: April 8, 2013). Study procedures followed the ethical standards of the Review Board and the Helsinki Declaration of 1975. All patients or their legally authorized representatives provided written informed consent prior to enrollment.

Subjects

The Ruby study, we enrolled critically ill adults with established KDIGO stage 2–3 AKI across 21 sites in Europe and the United States. We excluded patients with a prior kidney transplant, those receiving or in imminent need of RRT, those receiving comfort measures only, and those with known HIV infection or active viral hepatitis. The Sapphire study enrolled critically ill adults with cardiac or respiratory dysfunction without known stage 2–3 AKI at the time of enrollment from 35 sites in Europe and North America. Samples from a subset of Sapphire patients who developed stage 2–3 AKI within 1-week of enrollment were included in this present analysis. For this purpose, in the Sapphire cohort, serial biomarker measurements were included from the point at which patients met stage 2–3 AKI criteria, mirroring the time course of enrollment and biomarker measurement in the Ruby. We conducted a

complete case analysis, where in both studies, patients without uCCL14 concentrations for all three samples or who received RRT or died before the third sample was taken were excluded. Patients were recruited based on AKI assessment using creatinine and urine output criteria by local site investigators and all patients enrolled were included on an "intention to diagnose" basis (10, 14).

Sample Collection and Testing

In the Ruby study, urine samples were collected bid for 3 days from enrollment and then once daily for 4 days. In the Sapphire study, urine samples were collected bid for 4 days from enrollment and then once daily for 3 days. In both studies, urine samples were centrifuged, flash frozen, stored at or below –70°C, and thawed prior to sample testing. The primary analysis used uCCL14 concentrations from the first three scheduled urine collections in Ruby and the first three urine collections after onset of KDIGO stage 2 or 3 AKI in Sapphire for each patient. Technicians who were blinded to the clinical data measured uCCL14 concentrations in the samples using the NEPHROCLEAR uCCL14 Test on the Astute 140 Meter (Astute Medical, San Diego, CA).

Clinical Endpoint

The primary endpoint for the present analysis was the development of PS-AKI, as described (10, 12). In brief, patients who developed 72 consecutive hours of stage 3 AKI, commencing within 48 hours of first sample collection, or those who died following stage 3 AKI or received RRT prior to 48 hours from first sample collection or within 72 consecutive hours of stage 3 AKI, were considered endpoint positive. For the purposes of determining the primary endpoint, in Ruby, each patient's baseline serum creatinine was retrospectively adjudicated by a physician blinded to biomarker data based on serum creatinine data collected from the medical record from 6 months prior to 3 months after enrollment (9). In Sapphire, the reference values for serum creatinine were obtained as follows: if at least five values were available, the median of all values available from 6 months to 6 days prior to enrollment was used. Otherwise, the lowest value in the 5 days prior to enrollment was used. If no pre-enrollment creatinine was available, the creatinine value at the time of enrollment was used (10, 14).

Statistical Analysis

We stratified the uCCL14 concentrations in the first three urine collections into three levels: low (≤ 1.3 ng/mL), medium (> 1.3 to ≤ 13 ng/mL), and high (> 13 ng/mL), using previously validated clinical risk cutoffs (13). The high sensitivity cutoff at 1.3 ng/mL uCCL14 was derived to identify the majority of patients at risk of PS-AKI and the high specificity cutoff at 13 ng/mL to identify the highest risk patients where PS-AKI is most likely. To assess the validity of using a pooled dataset, we applied the Cochran Q test to assess heterogeneity of relative risk (RR) between individual time points across Ruby and Sapphire. Patients in the pooled analysis cohort were grouped by the pattern of uCCL14 levels across the three samples; these were then compared based on proportion of patients positive for PS-AKI within each group. Continuous and categorical variables were analyzed using the Wilcoxon rank-sum and Fisher exact tests, respectively. Two-sided p values of less than 0.05 were considered statistically significant. All analyses were performed using R 4.0.2 (R Foundation for Statistical Computing. Vienna, Austria).

Sensitivity Analyses

In addition to primary complete-case analysis, we performed a sensitivity analysis including all individuals with one or more uCCL14 measurements after enrollment to assess consistency of our findings in those with missing values for clinical reasons including discharge, death, and anuria. Finally, to test uCCL14 in the context of expected clinical use, our primary analysis utilized previously derived clinical cutoffs to categorize uCCL14 levels and changes. As an alternative, we performed a category-free analysis of absolute uCCL14 levels and any trend in the first 24 hours.

Extended Measurement of uCCL14

CCL14 measurement in urine samples collected at least daily allowed assessment of change in category of serial uCCL14 measurements over a longer time and its relationship to the occurrence of PS-AKI on day 7. We then performed rolling assessment of the PS-AKI composite endpoint over time, that is, at each time point of measurement, the occurrence of 72 hours of sustained stage 3 AKI commencing within 48 hours or death or

RRT with 48 hours of that measurement was assessed. For this exploratory analysis, we treated uCCL14 concentration as a continuous variable and explored discrimination of PS-AKI over time. Ability of uCCL14 concentration at any time point to discriminate the rolling endpoint was assessed as area under the receiver operating characteristic curve (ROC-AUC) with 95% CI determined by the DeLong method (15).

RESULTS

Patients

The Ruby study recruited 364 patients within 36 hours of clinical diagnosis of stage 2–3 AKI, while of the 723 patients critically ill patients without AKI stage 2 or greater in SAPPPHIRE analysis cohort, 212 then developed stage 2–3 AKI and were eligible for inclusion in this pooled analysis from that time point. After further exclusion of patients without three consecutive measurements of uCCL14 after meeting enrollment criteria, a total of 417 (268 patients from the Ruby study and 149 patients from Sapphire) were included in our complete-case analysis (**Fig. 1**). In addition, a further 111 patients had one or two valid uCCL14 measurements within 36 hours of enrollment and were included in a sensitivity analysis of 528 patients. Of the pooled population, 75 (18%) reached the primary endpoint of PS-AKI, while in the sensitivity analysis, 135 (26%) reached the endpoint, often reflecting the effect of early death or anuria preventing collection of a full set of study samples. Of the 111 patients in the sensitivity analysis but not the analysis cohort, 33 (30%) were receiving RRT, 18 (16%) were anuric, and 8 (7%) died. In the Sapphire cohort, only 7.4% of patients reached the primary endpoint compared with 24% in the Ruby study (**Table 1**), reflecting lower overall severity of AKI in the Sapphire cohort, AKI stage 3 at time of first sample collection 4% versus 30%. Conversely, the Sapphire cohort had higher nonrenal Acute Physiology and Chronic Health Evaluation II score (62 vs 52) and use of mechanical ventilation (85 vs 54%) (**Tables S1** and **S2**, http://links.lww.com/CCX/B147). In the pooled primary dataset, the median age was 65 years and 59% were male (Table 1). Baseline kidney function, history of CKD, demographics, illness severity, and organ support did not significantly differ between patients with PS-AKI endpoint and those without. However, fluid balance on the first day after enrollment was significantly more positive in those who developed PS-AKI (2.3 vs 3.4 L; $p = 0.04$). Importantly, despite the differences between the studies, the RRs analysis showed that they behaved similarly across the uCCL14 strata at each time point, justifying the pooled cohort analysis (**Table S3**, http://links.lww.com/CCX/B147).

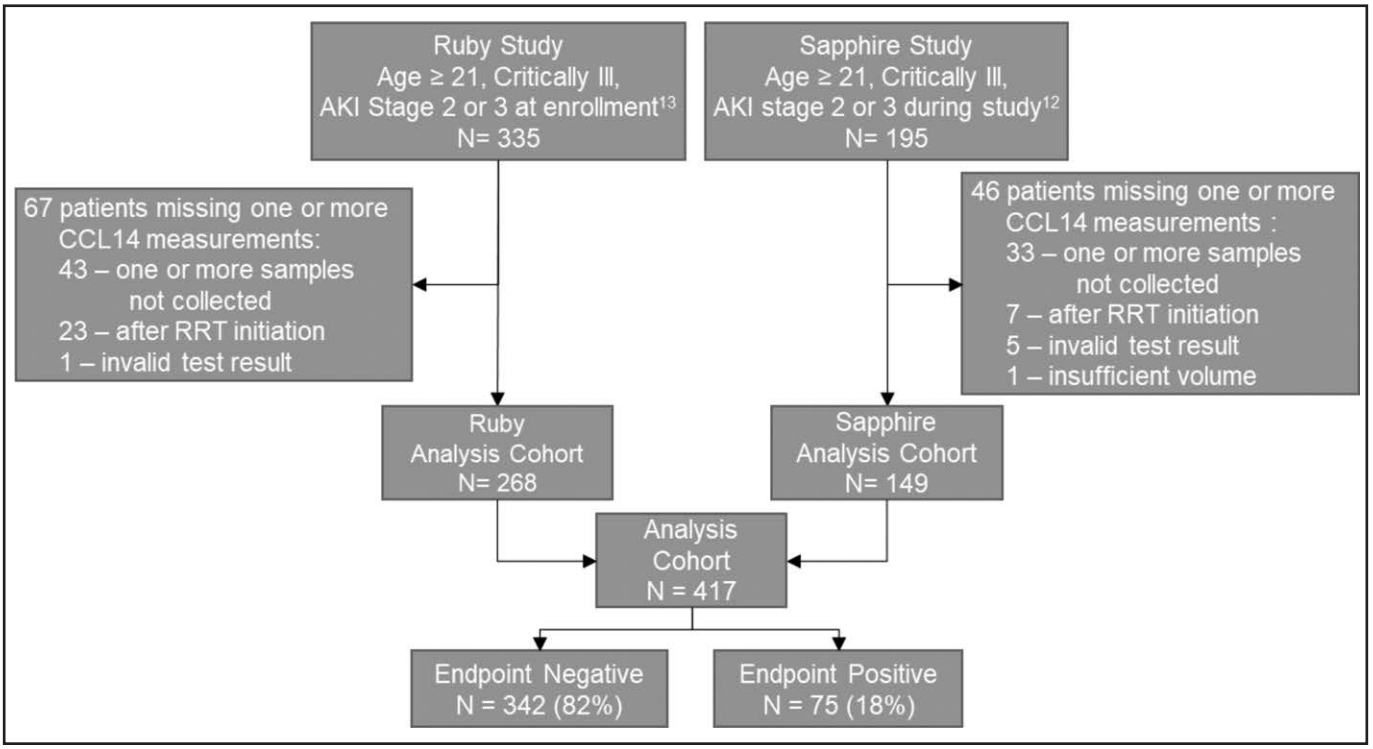

Figure 1. Patient flow diagram. AKI = acute kidney injury, CCL14 = C-C motif chemokine ligand 14, RRT = renal replacement therapy.

TABLE 1.
Baseline Characteristics of Analysis Cohort

Characteristics	All Patients	PS-AKI Endpoint Negative	PS-AKI Endpoint Positive	*p*
n	417	342	75	
Male, *n* (%)	248 (59)	201 (59)	47 (63)	0.604
Age[a] (yr)	65 (55–73)	65 (55–74)	64 (56–69)	0.107
Body mass index[a] (kg/m^2)	30 (26–36)	31 (26–37)	28 (25–34)	0.138
Race, *n* (%)				
Black or African American	50 (12)	43 (13)	7 (9)	0.615
Other/unknown	31 (7)	24 (7)	7 (9)	
White or Caucasian	336 (81)	275 (80)	61 (81)	
Chronic comorbidities, *n* (%)				
Chronic kidney disease	58 (14)	42 (12)	16 (21)	0.063
Diabetes mellitus	155 (37)	128 (37)	27 (36)	0.895
Heart failure	99 (24)	82 (24)	17 (23)	0.882
Coronary artery disease	144 (35)	121 (35)	23 (31)	0.503
Hypertension	289 (69)	239 (70)	50 (67)	0.583
Chronic obstructive pulmonary disease	81 (19)	66 (19)	15 (20)	0.873
Cancer	108 (26)	89 (26)	19 (25)	1.000
Reason for ICU admission, *n* (%)				
Respiratory	150 (36)	121 (35)	29 (39)	0.597
Surgery	136 (33)	117 (34)	19 (25)	0.173
Cardiovascular	176 (42)	136 (40)	40 (53)	0.039
Sepsis	98 (24)	77 (23)	21 (28)	0.367
Neurologic	27 (6)	24 (7)	3 (4)	0.443
Trauma	11 (3)	11 (3)	0 (0)	0.226
Other	107 (26)	89 (26)	18 (24)	0.772
Baseline serum creatinine[a] (mg/dL)	1.0 (0.7–1.2)	1.0 (0.7–1.2)	1.0 (0.8–1.4)	0.137
Baseline estimated glomerular filtration rate	76 (54–95)	76 (55–95)	76 (53–96)	0.319
Nonrenal Acute Physiology and Chronic Health Evaluation III score at enrollment	55 (43–74)	56 (42–73)	55 (45–79)	0.379
Nonrenal Sequential Organ Failure Assessment score on study day 1	6 (4–9)	6 (4–9)	6 (5–10)	0.204
Time difference (in hr) between second and first samples[a]	11 (9–14)	11 (9–14)	10 (6–13)	0.011
Time difference (in hr) between third and first samples[a]	24 (21–26)	24 (22–26)	22 (19–25)	0.005
Time difference (in hr) between third and second samples[a]	12 (11–13)	12 (11–13)	12 (11–13)	0.564
Fluid balance[a] (mL) on study day 1	2,426 (745–5,067)	2,300 (623–4,799)	3,373 (1,219–6,552)	0.040
Diuretic use on study day 1, *n* (%)	162 (39)	131 (38)	31 (41)	0.695
Vasopressor use on study day 1, *n* (%)	199 (48)	165 (48)	34 (45)	0.702

(*Continued*)

TABLE 1. (*Continued*)
Baseline Characteristics of Analysis Cohort

Characteristics	All Patients	PS-AKI Endpoint Negative	PS-AKI Endpoint Positive	*p*
Mechanical ventilation use on study day 1, *n* (%)	273 (65)	228 (67)	45 (60)	0.285
Kidney Disease Improving Global Outcomes stage at first sample collection[b], *n* (%)				
No acute kidney injury	51 (12)	51 (15)	0 (0)	< 0.001
Stage 1	49 (12)	49 (14)	0 (0)	
Stage 2	231 (55)	204 (60)	27 (36)	
Stage 3	86 (21)	38 (11)	48 (64)	
RRT within 7 d, *n* (%)	43 (10)	14 (4)	29 (39)	< 0.001
Death within 7 d, *n* (%)	29 (7)	19 (6)	10 (13)	0.024
RRT or death within 7 d, *n* (%)	69 (17)	31 (9)	38 (51)	< 0.001
Ruby patients, *n* (%)	268 (64)	204 (60)	64 (85)	< 0.001

PS-AKI = persistent severe acute kidney injury, RRT = renal replacement therapy.
[a]Median (interquartile range).
[b]Kidney Disease Improving Global Outcomes stage after retrospective readjudication of acute kidney injury (AKI) status.
Study PS-AKI endpoint was development of persistent stage 3 AKI lasting at least 72 hr commencing within 48 hr of first sample collection, or those who died following stage 3 AKI or those who received RRT prior to 48 hr from first sample collection or within 72 consecutive hr of stage 3 AKI.

Initial uCCL14 levels were low (≤ 1.3 ng/mL) in 196 (47%), medium (> 1.3 to ≤ 13 ng/mL) in 180 (43%), and high (> 13 ng/mL) in 41 patients (9.8%) (**Table 2**). As previous described (13), the initial uCCL14 category was strongly associated with development of PS-AKI (Table 2). Importantly, the association between uCCL14 category and PS-AKI outcome was essentially unchanged at the 12 and 24 hours time points (Table 2). In line with this observation, in the majority of cases (66%) uCCL14 category was unchanged from 0- to 24-hour time points and in only one patient did a two-level change occur (**Fig. 2**; and **Table S4**, http://links.lww.com/CCX/B147). General consistency of uCCL14 values over the first three measurements is visually demonstrated in **Figure S1** (http://links.lww.com/CCX/B147). Where changes occurred, they were generally consistent in direction with only 24 of 417 (6%) showing a fluctuating course in uCCL14 category (Table S4, http://links.lww.com/CCX/B147). In patients experiencing a change in uCCL14 category, we classified patients as decreasing or increasing uCCL14 category between 0 and 24 hours and stratified these by the initial uCCL14 levels. Across all initial categories, a change in uCCL14 category was associated with a corresponding change in risk of PS-AKI (Fig. 2). We assessed the additional information provided by changes in uCCL14 level, accounting for the initial value in logistic regression. Accounting for initial uCCL14 category, a decrease in uCCL14 category in the first 24 hours was associated with decreased risk of PS-AKI (odds ratio [OR], 0.2; 95% CI, 0.08–0.45; $p < 0.001$). An increase in category was associated with increased risk (OR, 4.04; 95% CI, 1.75–9.46; $p = 0.001$). When tested, there was no significant interaction between initial status and change ($p = 0.36$ for interaction term) (**Table 3**).

Sensitivity Analysis

Change in uCCL14 remained statistically significant when considered as a continuous variable in logistic regression including initial uCCL14 as a continuous log-transformed variable with comparable discrimination compared with our model based on category and change in category supporting the additional information provided by change in uCCL14 over serial measurement (**Table S5**, http://links.lww.com/CCX/B147).

In sensitivity analysis including patients with missing uCCL14 values, the association between change in uCCL14 stratified by baseline was essentially

TABLE 2.

C-C Motif Chemokine Ligand 14 Concentrations at Three Time Points in the First 24 Hours, Categorized by Predefined C-C Motif Chemokine Ligand 14 Cutoff and the Study Persistent Severe Acute Kidney Injury Endpoint

Sampling Time Points	All Patients	PS-AKI Endpoint Negative, *n* = 342	PS-AKI Endpoint Positive, *n* = 75	*p*
First sample (0 hr)				
Median of all results (IQR)	1.44 (0.60–4.53)	1.12 (0.52–2.79)	7.29 (4.03–24.49)	< 0.001
≤ 1.3 ng/mL, *n* (%)	196 (47)	186 (54)	10 (13)	< 0.001
> 1.3 and ≤ 13 ng/mL, *n* (%)	180 (43)	143 (42)	37 (49)	
> 13 ng/mL, *n* (%)	41 (10)	13 (4)	28 (37)	
Second sample (12 hr)				
Median of all results (IQR)	1.05 (0.47–3.94)	0.82 (0.40–2.30)	5.97 (3.10–21.38)	< 0.001
≤ 1.3 ng/mL, *n* (%)	228 (55)	221 (65)	7 (9)	< 0.001
> 1.3 and ≤ 13 ng/mL, *n* (%)	148 (35)	106 (31)	42 (56)	
> 13 ng/mL, *n* (%)	41 (10)	15 (4)	26 (35)	
Third sample (24 hr)				
Median of all results (IQR)	0.99 (0.44–3.12)	0.78 (0.38–1.82)	7.85 (2.31–19.64)	< 0.001
≤ 1.3 ng/mL, *n* (%)	231 (55)	221 (65)	10 (13)	< 0.001
> 1.3 and ≤ 13 ng/mL, *n* (%)	143 (34)	107 (31)	36 (48)	
> 13 ng/mL, *n* (%)	43 (10)	14 (4)	29 (39)	

IQR = interquartile range, PS-AKI = persistent severe acute kidney injury.

unchanged apart from overall worse outcomes for "unchanged" values reflecting inclusion of patients where measurement was truncated by death or need for RRT (**Fig. S2**, http://links.lww.com/CCX/B147), with a similar pattern of association in the logistic regression (**Table S6**, http://links.lww.com/CCX/B147).

Extended Measurement of uCCL14

uCCL14 measurements were conducted at least daily up to 7 days after enrollment in surviving patients remaining in ICU with urinary catheter and without RRT. At 36 hours, 355 patients of the original 417 had measurements available, which progressively declined to 148 by day 7. Temporal changes in uCCL14 categorization and outcomes are presented in **Figure 3**. Compared with the original value over time, uCCL14 values tended to decrease more than increase (Fig. 3 and **Fig. S3**, http://links.lww.com/CCX/B147) and 67% of patients were in the low category of uCCL14 at their last measurement (Fig. 3). Conversely, those with a last measured uCCL14 that remained in the high category were more likely to have died or received RRT by day 7 (Fig. 3). Overall, 75% of those who had died or received RRT by day 7 were in the medium or high category of uCCL14 at their last available measurement (Fig. 3). Rolling assessment of ability to predict the PS-AKI composite endpoint commencing within 48 hours demonstrated sustained ability to well-discriminate the endpoint with ROC-AUC greater than 0.8 through 7 days (**Fig. S4**, http://links.lww.com/CCX/B147).

DISCUSSION

In the current study, we demonstrate important clinical features of the previously described and validated uCCL14 assay that will both guide clinicians in its interpretation and provide additional confidence to its interpretation in real-world clinical settings. Importantly, we used a standardized uCCL14 assay that has been developed and approved for routine clinical use in Europe (16). We demonstrate that in two-thirds of patients with moderate to severe AKI, uCCL14 category remained unchanged over a 24-hour period, and where changes occurred, this indicated a corresponding change in risk level for PS-AKI.

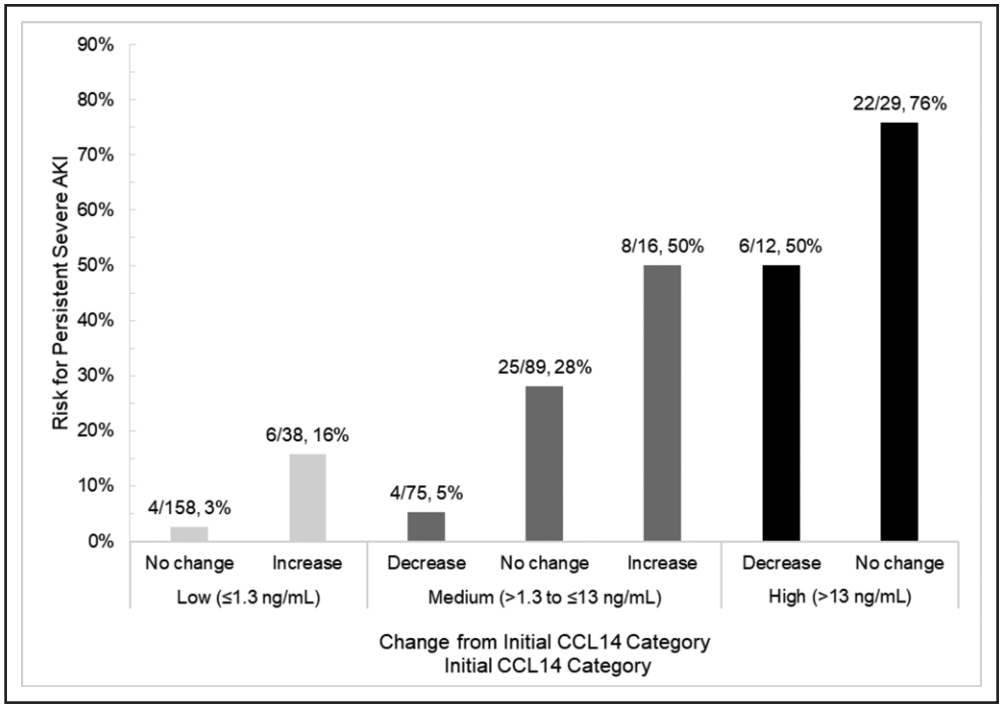

Figure 2. Urinary C-C motif chemokine ligand 14 (uCCL14) category changes stratified by initial uCCL14 category after the diagnosis of moderate to severe acute kidney injury (AKI) complete case analysis. Overall, 66% of patients had no change in C-C motif chemokine ligand 14 (CCL14) category in the first 24 hr after enrollment.

There are two important clinical implications of our findings. First, even if measurement of uCCL14 is delayed after first diagnosis of stage 2–3 AKI, the result is likely to provide good prognostication of clinical course of AKI as that provided by the original clinical investigations that derived and validated the assay. This suggests uCCL14 is an indication of a relatively persistent biological process within injured kidneys. Second, the serial measurement of uCCL14 may modify the assessment of risk over time. Notably, although uCCL14 category overall was generally unchanged, 51% of those with an initial medium value experienced a shift in category within the first 24 hours. Thus, for patients with an initial measurement in the medium risk category, serial measurement provides an opportunity to update risk assessment with relatively frequent and clinically important changes possible. Conversely, for patients with an initial uCCL14 in the high-risk category, a change to a lower category, while being associated with a clear reduction in risk, was still associated with a greater than 50% occurrence of the primary endpoint. Finally, it appears that uCCL14 retains ability to discriminate the composite endpoint up to 7 days after initial diagnosis of moderate to severe AKI, suggesting uCCL14 is potentially a flexible tool that could be used to assess underlying kidney pathophysiology through the time course of AKI. This work, therefore, represents an important contribution to defining the clinical utility and implementation of the uCCL14 assay for the identification of those at risk for PS-AKI, giving clinicians confidence in its interpretation over time.

The ability to categorize patients as a high or low risk of PS-AKI, RRT, or death has the potential to inform clinical decision-making and refine patient care. Guideline-based AKI care is often variably applied owing to insufficient prognostic information (17–19). Uncertainty regarding risk-benefit of interventions may explain the failure of many interventional trials

TABLE 3.

Logistic Regression Model for Study Persistent Severe Acute Kidney Injury Endpoint With Initial C-C Motif Chemokine Ligand 14 Category and Change in C-C Motif Chemokine Ligand 14 Category Over the First Day As Predictor Variables—Primary Analysis Cohort

Variable	OR (95% CI)	*p*
Initial category: medium	9.91 (4.64–23.3)	< 0.001
Initial category: high	107 (39.0–326)	< 0.001
Change in category: decrease	0.20 (0.08–0.45)	< 0.001
Change in category: increase	4.04 (1.75–9.46)	0.001

OR = odds ratio.

Reference levels: initial category: Low and change in category: unchanged.

Interaction term was eliminated from the model as there was no statistically significant interaction between initial category and change in category.

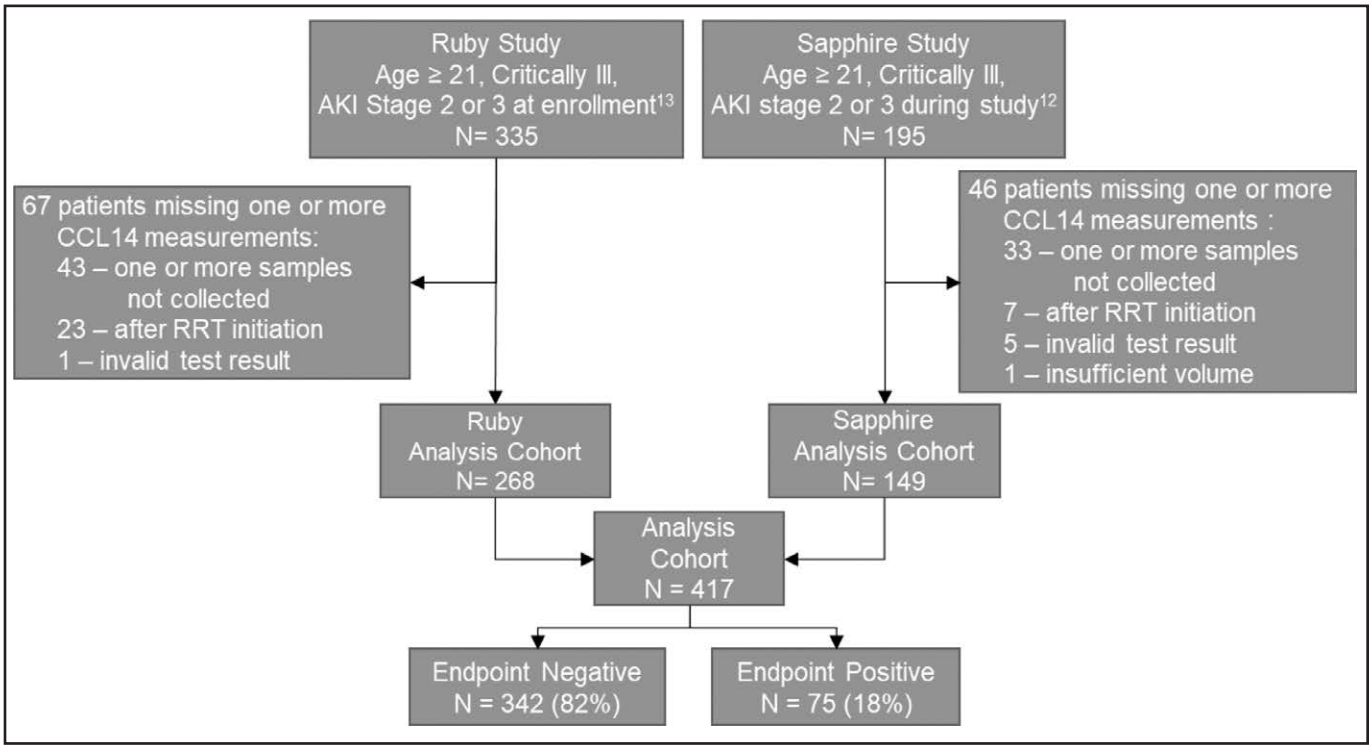

Figure 3. Alluvial plot demonstrating initial urinary C-C motif chemokine ligand 14 (uCCL14) category, first change in category (if any), final category, and outcome within 7 d for uCCL14 categories assessed serially over up to 7 d. AKI = acute kidney injury, CCL14 = C-C motif chemokine ligand 14, RRT = renal replacement therapy.

in AKI to improve outcomes (19–21). In this context, the ability of uCCL14 to provide consistent prognostic information over time could facilitate targeted trial inclusion in strategies for the treatment or prevention of PS-AKI.

Our study has both strengths and limitations. We studied a pooled cohort derived from two temporally separated studies of international mixed (medical/surgical) ICU populations, and as such, our findings are likely to be widely generalizable across critically ill populations and AKI etiologies. We studied patient-centered outcomes, death, need for RRT, and PS-AKI, which have been strongly linked to longer-term adverse patient-centered outcomes. We used the standardized assay implemented on an established, clinically available testing platform, which has now received Conformité Européene -marking for marketing within Europe. While the use of patients derived from two independent studies is a strength, there were much fewer cases of PS-AKI with Sapphire than Ruby, however, pooling appeared statistically appropriate (Table S3, http://links.lww.com/CCX/B147). Importantly, while patients were included based on clinical AKI stage 2 or greater, after expert adjudication of baseline creatinine, some patients in Ruby had less severe AKI, similarly in Sapphire, by the time of next biosampling, some patients no longer met AKI-2+. However, we believe our design represents a pragmatic assessment of a prognostic biomarker in a real-world clinical setting. Finally, observational studies cannot assess the benefit of uCCL14 measurement in directing clinical treatment decisions. Prospective clinical trials will be needed to examine the effect of differential treatment choices based on uCCL14 levels.

In conclusion, we have demonstrated the consistency of uCCL14 for the identification of patients at high risk for the development of PS-AKI across serial sampling and shown where changes occur these are paralleled in clinical outcomes.

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Drs. Prowle, Kampf, and Kellum devised the study developed the analysis plan and interpreted the results. Dr. Kwan performed the statistical analysis and developed the figures and tables with critical input from Drs. Prowle and Kampf. Dr. Prowle wrote the article. Drs. Prowle, Kampf, McPherson, and Kellum reviewed and developed the initial draft article. All authors reviewed the analysis plan and read and critiqued the article before final edits and approved the final submission.

The Ruby study (official title "Identification and Validation of Biomarkers of Acute Kidney Injury Recovery") and the Sapphire study (official title "Evaluation of Novel Biomarkers From Acutely Ill Patients at Risk for Acute Kidney Injury") were funded by Astute Medical (Astute Medical) a bioMérieux company.

Drs. Kampf, Kwan, and McPherson are employees of Astute Medical. The remaining authors have disclosed that they do not have any potential conflicts of interest.

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Full listing of Sapphire and Ruby investigators is provided in the ***Supplementary Material*** *(http://links.lww.com/CCX/B147).*

The Ruby study (official title "Identification and Validation of Biomarkers of Acute Kidney Injury Recovery") and the Sapphire study (official title "Evaluation of Novel Biomarkers From Acutely Ill Patients at Risk for Acute Kidney Injury") were approved by the Western Institutional Review Board (Puyallup, WA): Sapphire (Approval Number: 20101176; Date: August 27, 2010) and Ruby (Approval Number: 20130523; Date: April 8, 2013). All study procedures were followed in accordance with the ethical standards of the Review Board and with the Helsinki Declaration of 1975.

Informed consent was obtained from all individual participants included in the study or their legally authorized representatives.

Ruby study: https://clinicaltrials.gov/ct2/show/NCT01868724; Sapphire study: https://clinicaltrials.gov/ct2/show/NCT01209169.

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