Performance of a Standardized Clinical Assay for Urinary C–C Motif Chemokine Ligand 14 (CCL14) for Persistent Severe Acute Kidney Injury

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Key Points

- Using a standardized assay, we provide operating characteristics for two cutoffs for urinary C-C motif chemokine ligand 14 (CCL14) for the prediction of persistent severe AKI.
- A CCL14 cutoff of 1.3 ng/ml identifies 91% of patients who developed persistent severe AKI, need for RRT, or death, with a negative predictive value of 92%.
- In multivariable analyses, a CCL14 >13 ng/ml was associated with 10.4 adjusted odds for persistent severe AKI, need for RRT, or death ($P<0.001$).

Abstract

Background Clinical use of biomarkers requires the development of standardized assays and establishment of cutoffs. Urinary C-C motif chemokine ligand 14 (CCL14) has been validated to predict persistent severe AKI in critically ill patients with established AKI. We now report on the performance of standardized cutoffs using a clinical assay.

Methods A second aim of the multicenter RUBY Study was to establish two cutoffs for the prediction of persistent severe AKI (defined as KDIGO stage 3 AKI for at least 72 consecutive hours). Patients who received renal replacement therapy (RRT) or died before achieving 72 hours in stage 3 AKI were also considered to have reached the end point.

Results A cutoff value for urinary CCL14 of 1.3 ng/ml was determined to achieve high sensitivity (91%; 95% CI, 84% to 96%), and 13 ng/ml achieved high specificity (93%; 95% CI, 89% to 96%). The cutoff of 1.3 ng/ml identifies the majority (91%) of patients who developed persistent severe AKI with a negative predictive value of 92%. The cutoff at 13 ng/ml had a positive predictive value of 72% (with a negative predictive value of 75%). In multivariable adjusted analyses, a CCL14 concentration between 1.3 and 13 ng/ml had an adjusted odds ratio (aOR) of 3.82 (95% CI, 1.73 to 9.12; $P=0.001$) for the development of persistent severe AKI compared with those with a CCL14 ≤1.3 ng/ml, whereas a CCL14 >13 ng/ml had an aOR of 10.4 (95% CI, 3.89 to 29.9; $P<0.001$).

Conclusions Using a clinical assay, these standardized cutoffs (1.3 and 13 ng/ml) allow for the identification of patients at high risk for the development of persistent severe AKI. These results have immediate utility in helping to guide AKI patient care and may facilitate future clinical trials.

Clinical Trial registry name and registration number: Identification and Validation of Biomarkers of Acute Kidney Injury Recovery, NCT01868724

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Introduction
AKI remains a common clinical syndrome, occurring in as many as 25% of hospitalized patients and more than 50% of patients in the intensive care unit (ICU) (1). AKI, regardless of patient location, is associated with increased length of stay, cost of care, morbidity (including the development of CKD), and mortality (2–5). However, AKI often resolves spontaneously or with initial management (e.g., discontinuation of nephrotoxic drugs, fluid resuscitation), and specific actions may be delayed because of uncertainty regarding clinical course. Patients with persistent AKI (e.g., lasting ≥3 days) have been shown to be at increased risk for adverse outcomes compared with those with more transient, self-limiting AKI (6–8). Thus, the early identification of those at highest risk for persistent AKI should allow for patient-specific directed care in order to improve outcomes. Additionally, rapid recognition of this high-risk group could facilitate AKI clinical trial enrollment by helping to select those patients most likely to meet patient-centered end points (e.g., death, dialysis) and who may benefit most from novel AKI therapies.

In the RUBY Study (9), we reported on the performance of urinary C-C motif chemokine ligand 14 (CCL14) for the prediction of persistent (≥72 hours) Kidney Disease Improving Global Outcomes (KDIGO) stage 3 (severe) AKI (10). These results have been recently confirmed in two independent cohorts (11,12). However, before these results can be integrated into clinical practice, a clinical assay is required, along with cutoffs corresponding to specific operating characteristics (e.g., sensitivity, specificity, and positive and negative likelihood ratios). In this preplanned secondary analysis of the RUBY Study, we now report on the performance of a standardized clinical assay, the NEPHROCLEAR CCL14 test, along with the derivation and operating characteristics of two distinct cutoff values on the CCL14 receiver operating characteristic (ROC) curve for the prediction of persistent severe AKI. We chose one cutoff for high sensitivity and another for high specificity. We compared rates of renal replacement therapy (RRT) or death across CCL14 strata defined by the cutoffs over the first 90 days post enrollment. We also evaluated CCL14 cutoffs together with clinical variables from the RUBY Study and assessed whether there was added value of the biomarker results, as previously described (9). Secondary end points included RRT initiation, death, and the composite of RRT initiation or death within 90 days.

Sample Collection and Testing
In both the RUBY and reference population cohorts, urine samples were collected at enrollment and centrifuged. Supernatants were flash frozen within 2 hours of collection and stored at ≤−80°C. Thawed samples were analyzed for CCL14 using the NEPHROCLEAR CCL14 test on the Astute140 Meter (Astute Medical, San Diego, CA) by operators blinded to the clinical data. Concentration results for the NEPHROCLEAR CCL14 Test are traceable to reference material that contains defined mass of the CCL14 protein in accordance with EN ISO 17511 (14).

Cutoff Selection
Two cutoffs on the urinary CCL14 ROC curve were selected for risk assessment for development of persistent severe AKI. A lower CCL14 concentration cutoff was chosen to achieve high sensitivity with reasonable specificity to enable early recognition of the majority of patients who subsequently developed persistent severe AKI (15). This cutoff was chosen to identify patients who would be candidates for actions recommended as increasing in priority by the KDIGO Clinical Practice Guideline as risk increases for adverse consequences of AKI. The types of actions considered in determining the appropriate balance between sensitivity and specificity included consultation from experts (nephrology, pharmacy, intensive care, etc.), additional diagnostic workup and prioritized evaluation of need for higher-intensity monitoring (including hemodynamic/ fluid monitoring and possible ICU admission), or RRT. In selecting the cutoff, we assumed the test would not be used in a stand-alone manner as the sole basis for initiating invasive procedures, which should be ordered only after careful consideration of all clinical and laboratory test information, to ensure the potential benefits outweigh the risks. A second higher-concentration cutoff was selected to enable identification of patients who will develop persistent severe AKI with high specificity. This high specificity cutoff was selected to identify the subgroup of patients who are at the highest risk of persistent severe AKI and thus have the most urgent need for evaluation to receive actions recommended by the KDIGO Clinical Practice Guideline.

Materials and Methods
Subjects
The RUBY Study and the reference population study have both been described previously (9,13). RUBY enrolled adult ICU patients with established KDIGO stages 2–3 AKI across 21 sites in Europe and the United States from June 2013 to May 2014. Patients were excluded if they had a prior kidney transplant, were receiving or were in imminent need of RRT, were receiving comfort measures only, or had known infection with HIV or active hepatitis. The reference population study enrolled two cohorts of adult subjects at six sites from April 2012 to November 2012: (1) apparently healthy subjects and (2) subjects with prespecified stable chronic conditions without acute illness (13). Protocols for both studies were approved by Institutional Review Boards or ethics committees as required by each participating site, with all subjects (or their proxies) providing written informed consent.

Study End Point
The primary end point of the RUBY Study was persistent severe AKI, defined as KDIGO stage 3 AKI for at least 72 consecutive hours. Patients who received RRT or died before achieving 72 hours in stage 3 AKI were considered end point positive. Patients who were in stage 2 AKI at the time of enrollment who received RRT or progressed to persistent severe AKI starting within 48 hours were also considered end point positive (9,10). Reference serum creatinine (sCr) was determined by expert adjudication blinded to the biomarker results, as previously described (9). Secondary end points included RRT initiation, death, and the composite of RRT initiation or death within 90 days.
Importantly, values between these cutoffs are not “indeterminant,” nor do values in this range represent a "gray zone" (16). Instead, this portion of the ROC curve reflects a transition from high sensitivity to high specificity.

**Statistical Analyses**

Operating characteristics, including sensitivity, specificity, negative and positive predictive values, and negative and positive likelihood ratios, for the primary end point were calculated throughout the range of CCL14 concentrations to assess the clinical performance at different cutoffs. Differences in CCL14 concentrations among healthy subjects, those with chronic conditions, and RUBY subjects who did and did not develop the primary end point were presented in box and whisker plots. The Kruskal–Wallis test was used to detect differences in the concentrations among the four groups. Risk and relative risk for developing persistent severe AKI were calculated by dividing the cohort with the cutoffs. The reference stratum for relative risk consisted of those patients with CCL14 concentrations less than or equal to the lower (high sensitivity) cutoff. The Cochran–Armitage test was used to determine trend across risk strata defined by the cutoffs. The cumulative incidence curves for RRT initiation, death, and composite of RRT initiation or death within 90 days were estimated using the Kaplan–Meier method, and the log-rank test was used to compare groups defined by two cutoffs. To examine whether urinary CCL14 stratified by the two cutoffs improved risk prediction beyond clinical variables alone, a reference logistic regression model was constructed, as described previously (9). When analyzing CCL14 as a categorical variable in regression analyses, a CCL14 ≤ 1.3 ng/ml (the lower cutoff) was used as the reference level. Integrated discrimination improvement (IDI) and category-free net reclassification (cNRI) were used to assess the enhancement of the risk prediction by CCL14. Continuous, dichotomous, and polytomous baseline variables were compared across CCL14 strata by the Kruskal–Wallis, Cochran–Armitage, and Fisher’s exact tests, respectively. Confidence intervals for sensitivity, specificity, and positive and negative predictive values were calculated by the Clopper–Pearson exact method, whereas those for positive and negative likelihood ratio, risk, and relative risk were computed by the asymptotic method (normal approximation). Two-sided P values of <0.05 were considered statistically significant. Statistical analyses were performed using R v4.0.2 (R Foundation for Statistical Computing, Vienna, Austria), and IDI and cNRI were calculated using the “Hmisc” package (17).

**Results**

A cutoff value for urinary CCL14 of 1.3 ng/ml was determined to achieve high sensitivity (91%; 95% confidence interval [CI], 84% to 96%), and a cutoff of 13 ng/ml was found to achieve high specificity (93%; 95% CI, 89% to 96%), with the range in between reflecting a transition from high sensitivity to high specificity. Baseline characteristics for all RUBY study patients at enrollment are shown in Table 1 stratified by the two cutoffs (≤1.3, 1.3–13, and >13 ng/ml). Patients with higher CCL14 levels (>13 ng/ml) were less likely to have a history of coronary artery disease but more likely to be admitted to the ICU for respiratory failure or sepsis. A total of 211 (63%) patients had a CCL14 >1.3 ng/ml, whereas 54 (16%) had a value >13 ng/ml. Although there was no difference in the retrospectively adjudicated preadmission baseline sCr across the CCL14 strata, patients with a CCL14 concentration >13 ng/ml had significantly higher sCr at study enrollment compared with those with a CCL14 ≤1.3 ng/ml (P=0.011 between CCL14 ≤1.3 ng/ml and CCL14 >13 ng/ml). Using the previously described retrospective adjudication of baseline sCr (9), 55 patients (16% of the entire cohort) did not meet criteria for stages 2–3 AKI at the time of enrollment. Patients with higher CCL14 levels had more severe AKI at the time of study enrollment (P=0.001). Supplemental Table 1 provides the baseline characteristics for the cohort dichotomized by a CCL14 concentration higher or lower than 1.3 ng/ml.

Enrollment sCr was higher in those with elevated CCL14 concentrations and provided an area under the curve (AUC) of 0.81 for the primary end point of persistent severe AKI. Adding urinary CCL14 as a categorical variable stratified by the two cutoffs to enrollment sCr led to a significant increase in the AUC to 0.85 (P=0.02). We also compared the performance of 24-hour pre-enrollment urine output and CCL14 to predict the primary outcome. Supplemental Tables 2 and 3 demonstrate the poor performance of urine output to predict the primary outcome (AUC=0.63) and its nonsignificant odds ratio in a multivariate model (0.76; 95% CI, 0.57 to 1; P=0.05).

The operating characteristics from the RUBY Study for the two cutoffs and position of the cutoffs relative to the distributions of urinary CCL14 levels in the RUBY Study and reference population cohorts are shown in Figure 1. The cutoff of 1.3 ng/ml identifies the majority (91%) of RUBY Study patients who developed persistent severe AKI and has a high negative predictive value (92%) for this end point (with a positive predictive value of 47%). The cutoff at 13 ng/ml is higher than the vast majority (93%) of CCL14 values from RUBY Study patients who did not develop persistent severe AKI and has a high positive predictive value (72%) with a negative predictive value of 75%. Supplemental Table 4 provides the operating characteristics for urinary CCL14 values from 0.2 to 30 ng/ml in the RUBY Study population. Both cutoffs are substantially above the urinary CCL14 ranges for apparently healthy and stable chronic comorbidity reference cohorts.

Figure 2 shows the risk of developing persistent severe AKI across the three strata of CCL14 levels defined by the two cutoffs. The shading in each stratum shows the relative contribution of patients who met the persistent severe AKI composite end point by starting RRT, death, or persistently (>72 hours) elevated sCr or oliguria. Risk for the composite end point increased significantly with increasing CCL14 level, and the individual components, RRT and persistently elevated sCr or oliguria, were stratified similarly (P<0.001 for both). Increase in risk for death as a component of the primary end point was not statistically significant (P=0.13). Patients with a CCL14 concentration between 1.3 and 13 ng/ml were 4.8 (2.6–9) times as likely to develop persistent severe AKI compared with those with a CCL14 ≤1.3 ng/ml, whereas those with a CCL14 >13 ng/ml were
9 (median [IQR] 4.8–17) times as likely to develop persistent severe AKI. Risk of developing persistent severe AKI across the two strata of CCL14 levels defined by the 1.3 ng/ml cutoff are shown in Supplemental Figure 1.

Figure 3 illustrates the individual and combined rates of RRT and death in the cohort across CCL14 strata over the first 90 days post enrollment. Higher CCL14 values were associated with an increased risk of both death and RRT and the composite of the two (log-rank \(P<0.001\)). These findings persisted when the cohort was dichotomized at a CCL14 of 1.3 ng/ml (Supplemental Figure 2). Supplemental Table 5 demonstrates the median time (days from enrollment) for patients to receive RRT or die.

In order to evaluate the biomarker cutoffs in comparison with clinical variables, we performed a multivariable logistic regression analysis with persistent AKI as the end point with and without CCL14 as a covariate (Table 2). The results demonstrated that CCL14 concentrations were significant, even after accounting for clinical variables previously shown to be associated with persistent AKI (9). We found that the use of the two cutoffs added value to the clinical model using IDI and cNRI analysis (Supplemental Table 6). The addition of these CCL14 concentrations to the model significantly (\(P=0.02\)) increased the AUC from 0.86 (95% CI, 0.82 to 0.9) to 0.88 (95% CI, 0.85 to 0.92). Finally, as described above, we retrospectively adjudicated baseline creatinine for all patients. Using this

### Table 1. Baseline characteristics for all patients and stratified by the urinary C-C motif chemokine ligand 14 cutoffs at 1.3 and 13 ng/ml

|                      | All Patients | ≤1.3 ng/ml | >1.3 and ≤13 ng/ml | >13 ng/ml | \(P\) Value |
|----------------------|--------------|------------|-------------------|----------|-------------|
| Patients, n          | 335          | 124        | 157               | 54       |             |
| Men                  | 209 (62)     | 81 (65)    | 96 (61)           | 32 (59)  | 0.39        |
| Age, yr\(\^a\)       | 64 (55–73)   | 63 (55–71) | 65 (56–73)        | 62 (52–72) | 0.35        |
| BMI, kg/m\(^2\)\(\^a\) | 29 (23–35)   | 30 (26–36) | 29 (25–36)        | 28 (24–32) | 0.12        |
| Race                 |              |            |                   |          |             |
| Black                | 35 (10)      | 17 (14)    | 12 (8)            | 6 (11)   |             |
| Other/unknown        | 17 (5)       | 2 (2)      | 10 (6)            | 5 (9)    |             |
| White                | 283 (85)     | 105 (85)   | 135 (86)          | 43 (80)  | 0.07        |
| Chronic comorbidities|              |            |                   |          |             |
| CKD                  | 59 (18)      | 17 (14)    | 31 (20)           | 11 (20)  | 0.19        |
| Diabetes             | 110 (33)     | 42 (34)    | 53 (34)           | 15 (28)  | 0.52        |
| CHF                  | 73 (22)      | 32 (26)    | 32 (20)           | 9 (17)   | 0.12        |
| CAD                  | 118 (35)     | 56 (46)    | 49 (32)           | 13 (25)  | 0.003       |
| Hypertension         | 228 (68)     | 88 (72)    | 109 (69)          | 31 (57)  | 0.1         |
| COPD                 | 54 (16)      | 16 (13)    | 33 (21)           | 5 (9)    | 0.97        |
| Cancer               | 85 (25)      | 23 (19)    | 46 (30)           | 16 (30)  | 0.05        |
| Reason for ICU admission|           |            |                   |          |             |
| Respiratory          | 96 (29)      | 24 (19)    | 53 (34)           | 19 (35)  | 0.009       |
| Surgery              | 105 (31)     | 41 (33)    | 50 (32)           | 14 (26)  | 0.39        |
| Cardiovascular       | 149 (45)     | 54 (44)    | 69 (44)           | 26 (48)  | 0.62        |
| Sepsis               | 75 (22)      | 19 (15)    | 39 (25)           | 17 (32)  | 0.01        |
| Neurologic           | 17 (5)       | 8 (7)      | 6 (4)             | 3 (6)    | 0.6         |
| Trauma               | 7 (2)        | 3 (2)      | 2 (1)             | 2 (4)    | 0.8         |
| Other                | 109 (33)     | 39 (32)    | 53 (34)           | 17 (32)  | 0.9         |
| Vasopressors\(\^b\)  | 213 (64)     | 81 (65)    | 102 (65)          | 30 (56)  | 0.29        |
| Diuretics\(\^b\)     | 181 (54)     | 76 (61)    | 81 (52)           | 24 (44)  | 0.03        |
| Fluid balance, ml\(^c\) | 3271 (1285–6422) | 2267 (365–4219) | 4282 (1932–7419) | 3420 (1554–7447) | <0.001 |
| Days from ICU admission\(\^a\) | 1.1 (0.7–2.2) | 1.4 (0.8–2.8) | 1.1 (0.7–1.9) | 1.1 (0.6–1.9) | 0.11 |
| Mechanical ventilation| 187 (56)     | 65 (52)    | 96 (61)           | 26 (48)  | 0.99        |
| Baseline serum creatinine, mg/dl\(^a\) | 1 (0.8–1.2) | 1 (0.7–1.2) | 1 (0.8–1.3) | 1 (0.8–1.3) | 0.11 |
| Enrollment serum creatinine, mg/dl\(^a\) | 2.4 (1.7–3.3) | 1.7 (1.3–2.5) | 2.8 (1.9–3.6) | 3.2 (2.4–4.2) | <0.001 |

Data are presented as n (%) unless indicated otherwise. CCL14, C-C motif chemokine ligand 14; BMI, body mass index; CHF, congestive heart failure; CAD, coronary artery disease; COPD, chronic obstructive pulmonary disease; ICU, intensive care unit; KDIGO, Kidney Disease Improving Global Outcomes; APACHE, Acute Physiology, Age, Chronic Health Evaluation.
\(\^a\)Median (interquartile range).
\(\^b\)Vasopressors and diuretics are defined as any use from 3 days before through day 1 (day of study enrollment).
\(\^c\)Fluid balance is cumulative from the day before through the day of enrollment.
retrospective baseline creatinine to determine AKI stage, 55 patients were determined not to have stage 2 or 3 AKI at enrollment. Of these 55 patients, 37 (67%) had stages 2–3 AKI within the 24 hours before enrollment, but their AKI stage improved. We conducted a sensitivity analysis excluding these 55 patients. Supplemental Table 7 demonstrates that excluding these patients did not significantly change the odds ratio of either strata of CCL14 concentration (compared with Table 2). Additionally, Supplemental Figure 3 displays the operating characteristics for those subjects adjudicated to have stage 2 or 3 AKI at enrollment for the two cutoffs and position of the cutoffs relative to the distributions of urinary CCL14 levels in this cohort and reference population cohorts. The operating characteristics remain similar to those displayed in the intention-to-diagnose cohort (Figure 1).

Discussion
We have derived and characterized two cutoffs for urinary CCL14 using a standardized clinical assay to aid in clinical risk assessment for the development of persistent severe AKI. Assay standardization is essential for clinical implementation and is required before cutoffs can be specified. These cutoffs were determined by optimizing the operating characteristics (sensitivity, specificity, and negative and positive predictive values) on the basis of the intended clinical use of the NEPHROCLEAR CCL14 test. A high sensitivity cutoff at 1.3 ng/ml CCL14 can be used to identify the vast majority of patients who will develop persistent severe AKI, and who thus are candidates for actions recommended as increasing in priority by the KDIGO Clinical Practice Guideline as risk increases for adverse consequences of AKI. A high specificity cutoff at 13 ng/ml can be used to identify the highest-risk patients who are in most urgent need of evaluation for further intervention. Clinical relevance of these two cutoffs is demonstrated by their ability to stratify risk for adverse events (RRT or death) over 90 days and to add significant predictive information over clinically available information. Importantly, although CCL14 has already been validated as a biomarker for persistent AKI (9,11,12), this is the first report validating specific cutoffs using a standardized CCL14 assay developed for routine clinical use. This work represents an important step in framing the clinical utility of CCL14 for the identification of those at risk for persistent severe AKI.

The ability to determine which patients will develop persistent severe AKI is crucial because these patients are at highest risk for the most adverse outcomes. A meta-analysis of 18 studies demonstrated that the duration of AKI was independently associated with higher risk of cardiovascular events and incident stage 3 or greater CKD (18). These findings have been replicated in more recent studies not included in this meta-analysis. For example, Bihorac and colleagues demonstrated that among patients with septic shock, persistent AKI—defined as lasting at least 48 hours that did not recover at the time of
discharge—was associated with more than a five-fold increased risk of 1-year mortality compared with no AKI, even after adjusting for clinical factors (7,8). Separately, Kellum and colleagues showed that there are several distinct recovery phenotypes after an episode of stage 2 or 3 AKI; however, those with relapsing AKI and/or no recovery are at highest risk for 1-year mortality (as high as 45%) (19). Using data from the ProCESS (Protociled Care for Early Septic Shock) trial, Peerapornratana and colleagues demonstrated that in patients with septic shock, AKI biomarkers intended to predict the development of impending AKI (tissue inhibitor metalloprotease 2 and IGF binding protein 7 and neutrophil gelatinase associated lipocalin [NGAL]) were inadequate when attempting to predict persistent AKI at 1 week (7). These data highlight that much of the research around AKI biomarkers has sought to identify signals that are elevated in the absence of changes in sCr and urine output. However, there are limited data around the use of biomarkers to predict persistent AKI after severe AKI is already present. The TRIBE-AKI study has similarly published on the association of early postoperative biomarkers to prediction duration of AKI after adult cardiac surgery. They demonstrated that urinary kidney injury molecule-1 (KIM-1) had an AUC (SEM) of 0.75 (0.03) for the prediction of AKI ≥7 days, whereas combining KIM-1 with urinary IL-18 only improved the AUC to 0.76 (0.03) (6). Thus, there is limited utility among existing AKI biomarkers to predict persistent AKI.

We have demonstrated that urinary CCL14 provides significant information about the likelihood of persistent AKI that cannot be inferred from existing laboratory tests such as sCr or clinical variables alone. In our multivariable model (Table 2), CCL14 remained statistically significant, with elevated values being associated with 10.4 times the odds of persistent AKI. Similarly, the IDI and cfNRI were both statistically significant (P<0.001 for both; Supplemental Table 6). Therefore, the test provides important new information that can be used in conjunction with existing lab tests and clinical assessment to provide the most comprehensive view of a patient’s acute kidney status and likely course without further intervention. The high negative predictive value (92%) at the cutoff of 1.3 ng/ml ensures that almost all patients who test negative will not develop persistent AKI. Additionally, a value >1.3 ng/ml relates to a nearly one in two risk of developing persistent AKI (positive predictive value of 47%). At the higher cutoff (>13 ng/ml), the positive predictive value increased further to 72%, which is in line with the positive predictive value performance of other biomarkers such as B type natriuretic peptide (100 pg/ml had positive predictive value of 79% for acute heart failure) and high-sensitivity troponin I (>30 pg/ml had a positive predictive value of 75% for acute coronary syndrome) (20,21).

The ability to detect persistence early in a course of severe AKI has the potential to have a major clinical effect in the care of critically ill patients. There is an increasing body of literature demonstrating that patients with established AKI do not often receive guideline-based care (22–24). In multiple large-scale retrospective single-center cohorts and one multicenter international cohort, patients with known KDIGO AKI continued to receive nephrotoxic drugs (including nonsteroidal anti-inflammatory drugs and aminoglycosides) and experienced persistent hypotension (e.g., mean arterial pressures <55 mmHg), even in the setting of established stage 2 AKI (22,23). One of the most significant barriers to delivering these recommended clinical actions is that most AKI resolves spontaneously or with initial clinical management, and it is quite difficult to know which patients will require discontinuation of nephrotoxic drugs or a more intensive assessment of hemodynamics/volume status. Although biomarkers of early AKI and electronic alerts/care bundles have shown promise (25,26),
unfortunately, not all trials have provided improved outcomes (24,27,28). It is our hypothesis that a tool such as CCL14 that can better predict persistent severe AKI can serve as a tool to determine which patients need additional kidney-focused care beyond early management because it stands to reason that those destined for persistent severe AKI are most likely to benefit from strict adherence to kidney care bundles. For example, consider an ICU patient who develops early stage 1 AKI. A clinician reviews their medication list and assesses their volume status. They determine that the patient needs an additional bolus of balanced intravenous fluids, adjustment of vancomycin.

Table 2. Multivariable logistic regression model using clinical variables for prediction of persistent severe AKI without (reference model) and with (new model) urinary C–C motif chemokine ligand 14 as a categorical variable, with three levels stratified by two cutoffs at 1.3 and 13 ng/ml

| Variable                     | Reference Model | New Model with Urinary C–C Motif Chemokine Ligand 14 |
|------------------------------|-----------------|------------------------------------------------------|
|                              | Odds Ratio      | P Value                                              | Odds Ratio      | P Value                                              |
| BMI                          | 0.79 (0.54–1.13) | 0.21                                                 | 0.81 (0.54–1.18) | 0.29                                                  |
| Nonrenal APACHE III score    | 1.47 (1.1–2)    | 0.01                                                 | 1.38 (1.01–1.92) | 0.05                                                  |
| Serum creatinine trajectorya | 1.54 (1.15–2.11) | 0.005                                                | 1.47 (1.07–2.05) | 0.02                                                  |
| KDIGO stage at enrollment    | 5.43 (3.56–8.62) | <0.001                                               | 4.45 (2.86–7.2) | <0.001                                                |
| Diabetes                     | 0.56 (0.29–1.09) | 0.09                                                 | 0.5 (0.24–1.01) | 0.06                                                  |
| Urinary CCL14 >1.3 and ≤13 ng/ml | Not included | NA                                                    | 3.82 (1.73–9.12) | 0.001                                                 |
| Urinary CCL14 >13 ng/ml      | Not included    | NA                                                    | 10.4 (3.89–29.9) | <0.001                                                 |

For the categorical CCL14 variable, CCL14 ≤ 1.3 ng/ml was the reference level. All numeric variables were standardized by subtracting the mean and dividing by the standard deviation; N=312 (34% persistent). The clinical variables in the reference model were selected on the basis of an association with persistent severe AKI as described previously (9). CCL14, C–C motif chemokine ligand 14; BMI, body mass index; APACHE, Acute Physiology, Age, Chronic Health Evaluation; KDIGO, Kidney Disease Improving Global Outcomes; NA, not applicable.

*aChange in serum creatinine concentration over the prior day as determined using two serum creatinine results with mean±SD collection times of 18±9 and 7±4 hours before enrollment.
dosing on the basis of therapeutic monitoring, and to have piperacillin-tazobactam changed to cefepime. Now, consider if this patient progresses to stage 2 AKI, knowing that sCr changes often reflect injuries that have already occurred. The clinician will therefore be uncertain as to whether additional management changes are needed. If CCL14 is measured and is >1.3 ng/ml, then it may be appropriate to consider more aggressive clinical maneuvers (more intensive hemodynamic monitoring, stopping/starting other medications, or consulting nephrology). However, if the CCL14 is ≤1.3 ng/ml, then clinicians may be inclined to continue present management and make no further interventions.

Another clinical utilization of CCL14 could be around the initiation of RRT in the ICU. Recent large-scale randomized trials have sought to randomize patients with stage 2 or 3 AKI to accelerated initiation of RRT compared with standard care (29–33). However, in several of these trials, >30% of those randomized to standard care did not go on to develop a “hard” indication for RRT (29,30). The STARRT-AKI pilot trial and the ELAIN trial utilized elevated NGAL as part of the enrollment criteria, but several of the other trials did not use biomarkers (32,33). NGAL values were uniformly elevated in most patients and did not help discriminate those in need of RRT and those whose AKI would resolve without dialysis (33), whereas in our RUBY cohort, CCL14 results stratify patients with respect to initiation of RRT in the short term (Figure 2) or longer (Figure 3). Although lab tests alone are not expected to indicate which patient should or should not receive RRT, CCL14 may identify patients for whom additional clinical evaluation is appropriate in conjunction with medical history, physical examination, fluid balance, blood chemistry, and so on.

Our data show that CCL14 identifies patients with established AKI at high risk for adverse outcomes. This is not surprising, given the molecule’s roles in pathways involving leukocyte chemotaxis in the setting of tissue injury and repair (34). Because CCL14 is not expressed in mice or rats, there is little to no preclinical data on the role of CCL14 in the setting of AKI. Recent data demonstrate that in cell culture, exposure to TNFα led to alterations in CCL14 expression (35). Additionally, CCL14 has been shown to be a chemoattractant for monocytes and macrophages in the setting of inflammatory diseases such as systemic lupus and multiple sclerosis (36). As such, we postulate that in the setting of established AKI, CCL14 is subsequently released to recruit monocytes in order to help repair and rebuild the injured renal tissues. Further studies are needed to elucidate the role(s) of CCL14 in human AKI.

Our study has several important strengths and limitations. Our findings are strengthened by our enrollment of a large-scale international mixed (medical/surgical) ICU population, and thus our CCL14 findings are applicable to a diverse patient population with a variety of sources of AKI (nephrotoxic, inflammatory, ischemic, sepsis). Importantly, our findings were further strengthened through the use of a standardized assay that can be run on an established clinically available testing platform. A limitation is we only analyzed CCL14 at a single time point; analysis around serial testing was beyond the scope of this study. Furthermore, we were not able to analyze comparisons between CCL14 and other biomarkers of AKI with respect to performance of cutoffs because of lack of validated cutoffs for the other biomarkers for prediction of the study end point.

Although we were not able to evaluate the efficacy of using the CCL14 cutoffs to initiate specific clinical actions such as fluid resuscitation, medication changes, or RRT, we were able to adjust for a variety of clinical factors and demonstrated that CCL14 levels were still independently associated with the primary end point, thus providing actionable information about the patient’s kidney status. An additional strength of our study was analysis of patient-centered clinical outcomes (e.g., death or the receipt of RRT) because it demonstrates the clinical relevance of the derived cutoffs.

In summary, we have validated a standardized clinical assay for CCL14, the NEPHROCLEAR CCL14 test, and derived and characterized cutoffs for CCL14 using this test that allow for the identification of patients at high risk for the development of persistent severe AKI. These results have immediate utility in helping to guide patient care and may facilitate future clinical trials.

Disclosures
I.S. Chawla reports current employment by Silver Creek Pharmaceuticals; ownership interest in Exthera Medical, Lowell Therapeutics, and Stavro Medical; and patents and inventions with George Washington University. S. Demirjian reports research funding from National Institute of Diabetes and Digestive and Kidney Diseases (U01 DK129980-01); honoraria from Outset Medical; patents or royalties with Cleveland Clinic Innovation and the holder of US patent no. 10281455; and participating in a speakers’ bureau for Outset Medical. K.J. Gunnerson reports research funding from Spectral Medical. J.P. Kampf reports employment by Astute Medical, Inc. (a bioMérieux company) and ownership interest in bioMérieux. J.A. Kellum reports current employment by Spectral Medical; consultancy agreements with AM Pharma, Astellas, Astute Medical, Baxter, bioMérieux, Cytosorbents, Grifols, Klotho, Mallinckrodt, NxStage, Photophase, Potrero, and RenalSense; ownership interest with J3RM, Klotho, Photophase, and Spectral Medical; research funding from Astellas, Astute Medical, Axto Bio, Baxter, bioMérieux, Bioparto, Cytosorbents, Grifols, and RenalSense, patents and inventions with Astute Medical, Cytosorbents, J3RM, Klotho, and Photophase; and is the editor of Critical Care Clinics of North America and on the editorial boards of Nephrology Dialysis Transplantation, Critical Care, Critical Care Medicine, and Blood Purification. J.L. Koyner reports consultancy for Astute Medical/bioMérieux, Baxter, Mallinckrodt, Novartis, and SeaStar; research funding from Astute Medical, Fresenius Medical, the National Institutes of Health, and Nxstage Medical; honoraria from Acute Disease Quality Initiative (ADQI), the American Society of Nephrology, CSCR, and ISICEM; being listed on a patent for Pi GST to detect severe AKI following cardiac surgery with Arguts Medical; and is on the editorial board of Clinical Journal of American Society of Nephrology (CJASN), American Journal of Nephrology, and Kidney360; is on the scientific advisory board for Guard Therapeutics, the NKF-National, and Novartis; and participated in a speakers’ bureau for NxStage Medical. T. Kwan reports current employment by Astute Medical, Inc. (a bioMérieux company). P. McPherson reports employment by Astute Medical, Inc. (a bioMérieux company) and ownership interest in bioMérieux. S.T. Wilber reports ownership interest in Merck & Co., Inc. All remaining authors have nothing to disclose.
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Author Contributions

A. Bihorac, L.S. Chawla, S. Demirjian, J.A. Frey, K.J. Gunnerson, L. Hodgson, J.A. Kellum, J.L. Koyner, R. Schroeder, and S.T. Wilber were responsible for the investigation; A. Bihorac, L.S. Chawla, S. Demirjian, J.A. Frey, K.J. Gunnerson, L. Hodgson, J.P. Kampf, J.A. Kellum, T. Kwan, P. McPherson, R. Schroeder, and S.T. Wilber were responsible for supervision; A. Bihorac, S. Demirjian, J.A. Frey, K.J. Gunnerson, L. Hodgson, J.P. Kampf, J.A. Kellum, T. Kwan, P. McPherson, R. Schroeder, and S.T. Wilber reviewed and edited the manuscript; L.S. Chawla and J.L. Koyner wrote the original draft of the manuscript; J.P. Kampf, J.A. Kellum, J.L. Koyner, and T. Kwan curated the data; J.P. Kampf, J.A. Kellum, J.L. Koyner, and P. McPherson were responsible for the methodology; J.A. Kellum and T. Kwan were responsible for project administration; J.P. Kampf, T. Kwan, J.L. Koyner and P. McPherson were responsible for formal analysis; P. McPherson was responsible for resources.

Data Sharing Statement

Partial restrictions to the data and/or materials apply: patient-level data cannot be shared due to the consenting process, but the data used to help generate the ROC curves can be shared (see Supplemental Table 2).

Supplemental Material

This article contains supplemental material online at http://kidney360.asnjournals.org/lookup/suppl/doi:10.34067/KID.0008002021/-/DCSupplemental

Supplemental Table 1. Table of baseline characteristics with patients dichotomized by C-C motif chemokine ligand 14 (CCL14) concentrations above and at or below 1.3 ng/ml.

Supplemental Table 2. Comparison of the area under the curve for pre-enrollment urine output (UO) with urinary CCL14 concentration for the development of persistent severe AKI.

Supplemental Table 3. Urine output and urinary CCL14 concentration in a logistic regression model for the development of persistent severe AKI.

Supplemental Table 4. Operating characteristics for CCL14 concentration cutoffs from 0.2 to 30 ng/ml for the primary end point, persistent severe AKI.

Supplemental Table 5. Time to secondary outcomes (RRT and death).

Supplemental Table 6. Net reclassification and integrated discrimination improvement analysis of the addition of urinary CCL14 stratified by two cutoffs at 1.3 and 13 ng/ml to the clinical model in Table 2. Event, persistent severe AKI; nonevent, not persistent severe AKI.

Supplemental Table 7. Multivariable logistic regression model using clinical variables for prediction of persistent severe AKI without (reference model) and with (new model) urinary CCL14 as a categorical variable with three levels stratified by two cutoffs at 1.3 and 13 ng/ml and excluding those found to not have stage 2 or 3 AKI at enrollment.

Supplemental Figure 1. Risk of persistent severe AKI stratified by CCL14 level below and above 1.3 ng/ml. Within each CCL14 stratum, the individual components of the composite end point are displayed. The relative risk of persistent severe AKI for those patients with a CCL14 level >1.3 ng/ml to those with a CCL14 level <1.3 ng/ml is 5.9 (95% confidence interval, 3.2 to 11).

Supplemental Figure 2. Cumulative incidence of RRT, death and RRT, or death within 90 days of enrollment in the RUBY Study stratified by CCL14 concentrations below and above 1.3 ng/ml. The number of patients with CCL14 concentrations below and above 1.3 ng/ml are 124 and 211, respectively. The log-rank test was used to compute the P value for the differences between the strata.

Supplemental Figure 3. Comparison of CCL14 concentrations in four populations: healthy (n=378), chronic conditions without acute illness (n=366), RUBY intention-to-diagnose full cohort (n=335, 225 of whom did not develop persistent severe AKI), and those adjudicated to have stage 2 or 3 AKI at enrollment (n=280, 170 of whom did not develop persistent severe AKI). Bottom and top whiskers represent the 10th and 90th percentiles of the CCL14 concentrations in that group, respectively. Bottom and top boxes represent the 1st and 3rd quartiles, respectively. Middle bar is the median. The horizontal dashed lines correspond to the 1.3 and 13 ng/ml cutoffs. P value computed using the Kruskal–Wallis test <0.001.

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