BRIEF REPORT

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Club Cell Secretory Protein–Derived Acute Respiratory Distress Syndrome Phenotypes Predict 90-Day Mortality: A Reanalysis of the Fluids and Catheter Treatment Trial

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OBJECTIVES: Club cell secretory protein (CC16) is a protein with potential utility as a lung-specific biomarker for acute respiratory distress syndrome. The purpose of this study was to characterize CC16 in plasma from patients enrolled in the Fluid and Catheter Treatment Trial (FACTT) to determine the prognostic value for patient outcomes in our subgroup of FACTT patients.

DESIGN: A secondary biomarker analysis of a prospective randomized-controlled trial. The primary outcome was area under the receiver operating characteristic (AUROC) of CC16 for prediction of 90-day mortality. Secondary outcomes included differences in mortality, length of stay, and ventilator-free days (VFDs) between patients with high and low CC16. Statistical analyses were performed with IBM SPSS Statistics.

SETTING: Single-center laboratory analysis.

SUBJECTS: Plasma samples from 68 FACTT subjects and 20 healthy controls.

INTERVENTIONS: CC16 was measured in patient plasma samples by enzyme-linked immunosorbent assay.

MEASUREMENTS AND MAIN RESULTS: Subjects were an average of 48 years old (SD, 16.7 yr old) and 51.5% male. AUROC analysis of CC16 on day 1 showed an area under the ROC curve of 0.78 for prediction of mortality (odds ratio, 1.011; 95% CI, 1.003–1.021) with an optimal cutoff value of 45 ng/mL. Patients in the low CC16 group (<45 ng/mL) had lower mortality (7.5 vs 50.0%; $p < 0.001$) and similar VFD (11.9 vs 13.2; $p = 0.638$). When stratified by CC16 concentration, there was no difference between mortality in the fluid liberal (36.4 vs 58.8%; $p = 0.256$) or conservative (4.3 vs 11.8%; $p = 0.366$) groups.

CONCLUSIONS: CC16 demonstrated an acceptable AUROC for prediction of patient mortality with a cut point of 45 ng/mL. Patients with high CC16 on day 1 had worse outcomes compared with those with low CC16, suggesting a prognostic role for this lung-specific biomarker.

KEY WORDS: acute lung injury; acute respiratory distress syndrome; biomarker; lung epithelial cell; phenotype; SCGB1A1

Laboratory-based biomarkers for prognostication and phenotype-based treatments have been proposed for the management of acute respiratory distress syndrome (ARDS) (1–3). In particular, because the heterogeneity of ARDS has been implicated as a cause for several negative studies of ARDS treatments, evaluating potential differences in treatment response based on unique ARDS phenotypes is an attractive strategy (1–3). Differential treatment response based on biomarker-based phenotypes to fluid management, statin therapy, and lung protective ventilation has been observed (1, 4, 5). To

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date, this work has focused on nonspecific inflammatory markers (1, 4, 5). Lung-specific biomarkers may provide an enhanced understanding of the pathophysiologic mechanisms of phenotype-based prognostication and treatment response.

Club cell secretory protein (CC16) is a protein secreted from epithelial cells in the small bronchioles previously reported to have diagnostic and prognostic potential for ARDS (6–8). CC16 is highly specific to lung pathologies because it is only found in significant quantity in lung tissue (9). The objective of this study was to determine whether CC16 concentration within 24 hours after randomization could predict 90-day mortality and response to conservative fluid strategy in the Fluid and Catheter Treatment Trial (FACTT) (10). We hypothesized patients with high CC16 concentrations at 24 hours would have significantly higher mortality and an improved mortality response to a conservative fluid regimen when compared with those with low CC16 concentrations.

MATERIALS AND METHODS

This study was a secondary analysis of FACTT. In brief, FACTT was designed to compare a conservative or liberal fluid strategy and hemodynamic monitoring with a central venous or pulmonary artery catheter in patients with ARDS. Inclusion criteria, exclusion criteria, and results have previously been reported (10). This study was approved by the Augusta University Institutional Review Board (Reference number: 1128838-6) on November 2021, and a waiver of informed consent was obtained. Procedures were followed in accordance with the Helsinki Declaration of 1975.

We measured plasma CC16 in 68 patients from FACTT. The primary outcome was the predictive value of CC16 concentration on day 1 for 90-day mortality measured by the area under the receiver operating characteristic (AUROC) curve. The secondary outcome evaluated the prognostic utility of high versus low CC16 concentration for fluid strategy treatment response. Other outcomes included 90-day mortality, ventilator-free days (VFDs), and length of stay (LOS) outcomes when stratified by CC16. VFD was defined as the number of days alive and without mechanical ventilation in the first 28 days after enrollment. To determine the optimal cutoff for high versus low CC16 concentration, AUROC analysis for 90-day mortality was performed, Youden index (YI) was calculated, and the highest value recorded. Patients were stratified by this value to evaluate secondary outcomes.

Biosamples

Plasma samples and coded data sheets from patients enrolled in FACTT were obtained from the National Heart, Lung, and Blood Institute's Biological Specimen and Data Repository Information Coordinating Center. Additional 20 healthy patient plasma samples were obtained from Innovative Research (Novi, MI) to serve as controls. Patient samples were stored frozen at –80°C. Plasma CC16 concentration was assessed in duplicates on days 0, 1, and 3 by enzyme-linked immunosorbent assay using kits purchased from R&D Systems (Minneapolis, MN) per the manufacturer's instructions.

Statistical Analysis

All statistical analyses were performed in IBM (Armonk, NY) Statistical Package for the Social Sciences Statistics Version 27.0. Figures were developed in GraphPad Prism (San Diego, CA). Statistical significance was assessed at an alpha of 0.05. Patient demographics were assessed using descriptive statistics. Continuous variables were assessed with Student *t* test or Mann-Whitney *U* test for parametric and nonparametric data, respectively. Categorical variables were assessed with chi-square analysis. To determine the diagnostic value of CC16 concentration for ARDS, AUROC was calculated on combined control and FACTT samples. Logistic regression was performed in a backward stepwise fashion. All clinical variables were placed into the original model. At each step, the variable with the highest *p* value was removed until all remaining variables had a *p* value of 0.1 or less. Multicollinearity was excluded with variance inflation factors for each variable and goodness-of-fit was assessed with the Hosmer-Lemeshow test.

RESULTS

Patient Characteristics

Plasma CC16 concentrations were measured for 68 patients (34 in fluid conservative and 34 in fluid liberal groups) from FACTT and 20 healthy controls. Baseline characteristics were similar between groups with the exception that subjects in the liberal fluid strategy arm had a higher baseline Acute Physiology and Chronic Health Evaluation III (APACHE III) score (102 vs 81; $p = 0.002$), positive end expiratory pressure (PEEP) (11 vs 8 cm H_2O; $p = 0.026$), and tidal volume (5.1 vs 5.9 mL/kg; $p = 0.032$)

TABLE 1.
Patient Characteristics and Outcome by Club Cell Secretory Protein Concentration and Treatment Randomization

Characteristic	CC16 Concentration			Fluid management Strategy		
	< 45 ng/mL (*n* = 40)	≥ 45 ng/mL (*n* = 28)	*p*	Conservative (*n* = 34)	Liberal (*n* = 34)	*p*
Age, yr, mean (SD)	45.4 (14)	52.5 (20)	0.085	48.0 (18)	48.6 (15)	0.903
Acute Physiology and Chronic Health Evaluation, mean (SD)	86.2 (26)	99.6 (29)	0.056	81 (23)	102 (29)	0.002
Positive end expiratory pressure, cm H_2O, mean (SD)	9.9 (5.6)	8.8 (3.6)	0.354	8 (3)	11 (6)	0.026
TV, mL, mean (SD)	470 (127.6)	438 (73.9)	0.257	459 (108)	454 (110)	0.881
TV, mL/kg, mean (SD)	5.3 (1.4)	5.8 (1.9)	0.231	5.1 (1.0)	5.9 (1.9)	0.032
Ratio of Pao_2 to Fio_2, *n* (%)						
≥ 300	2 (5.0)	2 (7.1)	0.883	3 (8.9)	1 (2.9)	0.844
200–299	6 (15.0)	5 (17.9)		6 (17.6)	5 (14.7)	
100–199	23 (57.5)	16 (57.1)		19 (55.9)	20 (58.8)	
≤ 99	9 (22.5)	5 (17.9)		6 (17.6)	8 (23.5)	
Conservative strategy (%)	42.5	39.2	0.218	-	-	-
Inciting factors **(%)**						
Pneumonia	57.5	78.6	0.117	58.8	73.5	0.305
Sepsis	30.0	35.7	0.793	29.4	35.3	0.796
Aspiration	32.5	39.3	0.613	41.2	29.4	0.447
Direct injury	77.5	85.7	0.535	76.5	85.3	0.539
Outcomes (%)						
Mortality (total cohort)	7.5	50	< 0.001	14.7	35.2	0.091
Mortality (high CC16)	-	-	-	36.4	58.8	0.256
Mortality (low CC16)	-	-	-	4.3	11.8	0.366
Mortality (liberal)	11.8	58.8	0.006	-	-	-
Mortality (conservative)	4.3	36.4	0.011	-	-	-
Hospital length of stay, d, mean (SD)	17.1 (11.5)	18.8 (14.0)	0.616	17.3 (13.6)	18.2 (11.7)	0.748
ICU length of stay, d, mean (SD)	9.9 (9.6)	11.3 (10.6)	0.588	8.5 (8.4)	12.5 (11.0)	0.094
Ventilator-free days at 28 d, mean (SD)	13.2 (10.1)	11.9 (10.7)	0.638	12.4 (10.6)	13.0 (10.3)	0.817
CC16 information						
Day 0[a]	70.1 (51.9)	90.9 (60.3)	0.132	71.4 (55.3)	85.9 (56.7)	0.292
Day 1[a]	27.7 (10.6)	118.5 (87.7)	< 0.001	53.8 (62.3)	76.3 (80.1)	0.200
Day 3[a]	60.3 (73.8)	91.6 (74.9)	0.092	72.2 (81.4)	74.3 (70.0)	0.909

CC16 = club cell secretory protein, TV = tidal volume.
[a]No differences observed in CC16 among days 1, 2, and 3 assessed with repeated measures analysis of variance.

(**Table 1**). Patient outcomes were similar between the conservative and liberal groups including 90-day mortality (14.7 vs 35.2%; $p = 0.091$) and ICU LOS (8.5 vs 12.5 d; $p = 0.094$); however, there was a nonsignificant trend toward better outcomes in the conservative group.

CC16 Characterization

When comparing the entire cohort of patients, average CC16 concentrations were not significantly different among days 0, 1, and 3. There was also no difference in CC16 concentration based on the presence of

ARDS contributing factors. When compared with 20 healthy patient samples, subjects from FACTT had higher CC16 concentration on all days. Upon AUROC analysis of healthy controls and FACTT subjects, day 0 CC16 had a high predictive value for ARDS with an AUROC of 0.982 (95% CI, 0.958–1.000) and an optimal cutoff value of 13.5 ng/mL (YI, 0.906) with 95.6% sensitivity and 95.0% specificity. Patients with 90-day mortality had over double the day 1 CC16 concentration than survivors (50.1 vs 109.9 ng/mL; p = 0.002). There was no difference for days 0 or 3 CC16 concentration.

Primary and Secondary Outcomes

Upon AUROC analysis of day 1 CC16 concentration in relation to 90-day mortality, CC16 had an AUROC of 0.78 (95% CI, 0.652–0.905) with an optimal cutoff value of 45 ng/mL (YI, 0.569), which remained similar when controlling for covariates (0.81 [95% CI, 0.70–0.92]) (**Fig. S1**, http://links.lww.com/CCX/B7). When subjects were stratified by high (≥45 ng/mL) and low (<45 ng/mL) CC16 concentrations, baseline characteristics were balanced between groups with a trend toward higher baseline APACHE III in the high CC16 group (100 vs 86; p = 0.056). Patients with high day 1 CC16 concentration had higher 90-day mortality (50 vs 7.5%; p < 0.001) (**Fig. 1*A***) and similar VFD (11.9 vs 13.2; p = 0.638). This remained similar in both the liberal and conservative fluid groups (**Fig. 1**, ***B*** and ***C***). There was no difference in ICU LOS between the groups. Upon logistic regression controlling for APACHE III, every 1-ng/mL increase in CC16 predicted a 1.0% increase in mortality (odds ratio, 1.010 [95% CI, 1.001–1.021]) (**Table S1**, http://links.lww.com/CCX/B7). When assessing the effect of CC16 concentration on response to fluid intervention, patients with low CC16 had little difference in 90-day mortality (**Fig. 1*E***), while patients in the high CC16 group had a nonsignificant trend toward lower mortality with conservative fluid treatment (**Fig. 1*F***).

DISCUSSION

To our knowledge, this is the first analysis of CC16 in the context of a prospective randomized-controlled trial of ARDS. In this study, plasma CC16 concentrations had similar AUROC for prediction of ARDS as seen in previous studies (0.86–0.91 ng/mL) (6, 7). In addition, CC16 concentration had a good predictive value for 90-day mortality with an optimal cutoff value of 45 ng/mL and relatively well-balanced baseline characteristics.

Due to the fibrotic progression of ARDS, early intervention is critical to prevent long-term morbidity and mortality (2). Previous studies have demonstrated early elevated serum CC16 concentration can assist in distinguishing an ARDS diagnosis from other etiologies with a range of 9.2–33 ng/mL (6–8, 11). Patients with chronic lung diseases including emphysema, chronic obstructive pulmonary disease, and smokers have a decreased CC16 concentration when compared with healthy patients, whereas patients with acute lung pathologies (e.g., pneumonia and ARDS) have an increased concentration from baseline (9). In this prospective study of ARDS patients, an optimal cutoff value within this range (13.5 ng/mL) on day 0 after randomization was determined. Stratification by day 1 CC16 concentration significantly correlated with and predicted a higher rate of 90-day mortality. These results are similar to what has been described in previous observational studies of CC16 in ARDS (6, 7). Lin et al (6, 7) reported that CC16 concentration correlated with ICU LOS, and survivors had significantly lower concentrations than nonsurvivors. They also observed that CC16 correlated with ARDS severity, as defined by Pao_2/Fio_2 ratio. Although CC16 had relatively poor prognostic performance in one study of ARDS patients with renal failure, this is likely due to CC16 being renally eliminated and may be mitigated by use of a biomarker panel to improve prediction, similar to other ARDS phenotyping evaluations (2, 7). Taken together, these results support further investigation of CC16 as a prognostic biomarker.

Individualized biomarker-guided treatment plans are a notable gap in ARDS knowledge. Previous studies have demonstrated differences in response to interventions including ventilator and fluid management strategies, statins, and corticosteroid therapy, demonstrating a role for biomarkers (1, 4, 5). In this study, patients with a high CC16 on day 1 had a significant mortality difference with similar baseline characteristics and proportions of each treatment arm. Interestingly, when patients were divided by intervention arm, there was a nonsignificant trend toward better response to conservative fluid treatment in the high CC16 arm when compared with the low CC16 arm. This finding demonstrates a potential differential response to fluid strategy stratified by baseline CC16;

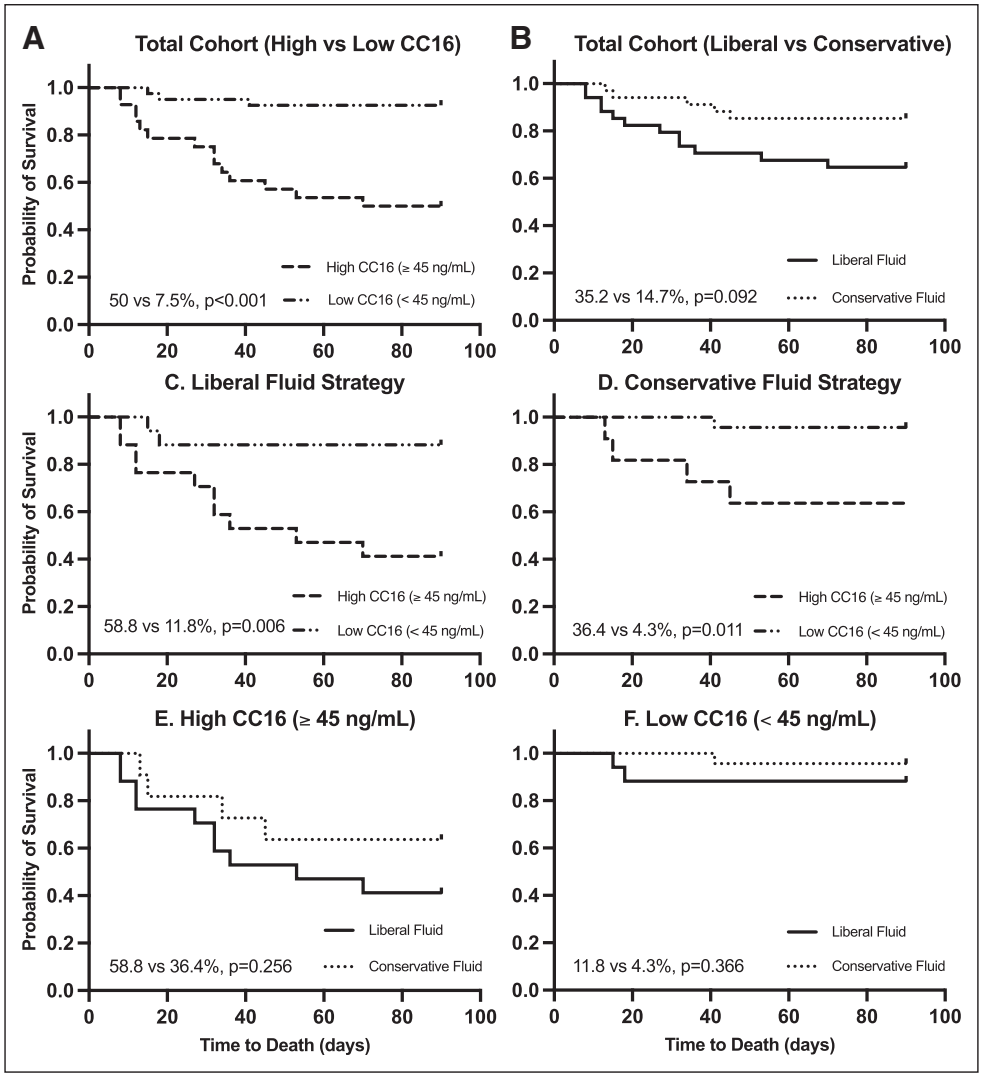

Figure 1. Kaplan-Meier survival curves stratified by CC16 concentration and fluid strategy. **A** and **B**, Total cohort ($n = 68$) stratified by Club cell secretory protein (CC16) concentration and fluid strategy. Patients with high CC16 had higher 90-d mortality. There was no difference when stratified by fluid strategy. **C** and **D**, Patients in the liberal ($n = 34$) and conservative ($n = 34$) fluid cohorts stratified by CC16. Those with high CC16 had higher mortality in both the liberal and conservative groups. **E** and **F**, Patients in the high ($n = 28$) and low ($n = 40$) CC16 groups stratified by fluid strategy. Those in the low CC16 group had a small difference in survival, while those in the high CC16 group had a stronger trend (both $p > 0.05$), potentially demonstrating a differential treatment response.

however, we were underpowered to observe the true difference. In previous studies, patients with cardiogenic pulmonary edema demonstrated a significantly higher plasma CC16 level and may benefit from conservative fluid management, explaining higher mortality trend in the high CC16 subgroup treated with a liberal fluid management strategy (11).

This study has several important limitations. First, its retrospective design precludes inferential conclusions. Furthermore, the small sample size may limit generalizability and potentially precluded observing treatment-related differences based on CC16 concentration. Some baseline differences (e.g., APACHE III and PEEP) were different between CC16 groups; however, in regression analyses, CC16 was still significantly associated with mortality. Finally, healthy patients were used as the non-ARDS control group (as opposed to critically ill, non-ARDS patients) when determining the diagnostic concentration for CC16. A previous study has reported a higher CC16 concentration (33 ng/mL) for diagnosis using critically ill non-ARDS patients, potentially decreasing the diagnostic accuracy in our cohort; however, the predictive values were still high in that study, suggesting good a diagnostic ability of CC16 (6–8, 11).

CONCLUSIONS

In the first analysis of a prospective, randomized trial of ARDS patients, CC16 concentration demonstrated acceptable ability to predict mortality and warrants future investigation as a lung-specific biomarker for ARDS prognostication.

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