Validation of Prognostic Club Cell Secretory Protein (CC16) Cut-point in an Independent ALTA Cohort

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ABSTRACT

BACKGROUND: Club cell secretory protein (CC16) has demonstrated utility as a lung-specific biomarker in predicting mortality in acute respiratory distress syndrome (ARDS). These findings have been observed in pre-clinical trials and a re-analysis of a large, randomized controlled trial of ARDS (Fluid and Catheter Treatment Trial (FACTT)).

OBJECTIVES: The purpose of this study was to validate previous findings by evaluating CC16 level as a mortality predictor in patients from the albuterol to treat acute lung injury (ALTA) trial.

DESIGN AND METHOD: In this secondary biomarker analysis, plasma CC16 level was measured from 100 ALTA subjects using enzyme-linked immunosorbent assay (ELISA). The rate of mortality was assessed in patients with high (⩾45 ng/mL) versus low CC16 (<45 ng/mL) levels. This cut-off level was applied based on our previous analysis from FACTT trial. Significance was assessed using Kaplan-Meier curves and a log-rank test.

RESULTS: Subjects were an average of 50 years old and 46% of them were females. Patients with high CC16 levels had higher 90-day mortality compared to those with low CC16 levels, (37.73% vs 8.95%, $P < .001$). Other clinical outcomes including ICU-free days, ventilator-free days, and organ failure free days were significantly different between the groups (All $P < .05$).

CONCLUSION: In this validation study, we demonstrated that ARDS patients with high plasma CC16 concentration had a higher mortality rate than those with low CC16 levels, confirming previous findings that CC16 levels are associated with ARDS mortality.

KEYWORDS: Acute lung injury, ARDS, lung epithelial cell, SCGB1A1, critical illness

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Introduction

Acute respiratory distress syndrome (ARDS) confers significant mortality and is often associated with poor prognosis, in part due to the lack of approved laboratory tests.[1] Previous analyses have evaluated non-specific biomarkers and have shown some success in diagnosis and prognosis; however, no ARDS-specific prognostic biomarker exists.

Club cell secretory protein (CC16) is a lung-specific protein largely secreted from epithelial cells in the small bronchioles of the lungs making it highly specific to lung pathologies.[2] In both pre-clinical and clinical studies of ARDS, CC16 has demonstrated its diagnostic and prognostic potential.[3-5] Recently, CC16-derived phenotypes have demonstrated utility in predicting 90-day mortality in a secondary analysis of the Fluid and Catheter Treatment Trial (FACTT), with an optimal cut-point of 45 ng/mL for stratification.[6] The objective of this study was to externally validate our previously described cut-point in an independent cohort of 100 patient samples from the albuterol to treat acute lung injury (ALTA) trial. We hypothesized that patients in the high CC16 group (>45 ng/mL) would have significantly worse outcomes than patients with low CC16.

Materials and Methods

In this secondary biomarker analysis, plasma CC16 concentration was measured from 100 ALTA subjects using an ELISA kit (R&D Systems, Catalog # DY4218, Minneapolis, MN) according to the manufacturer's instructions. The primary outcome was the difference in 90-day mortality between subjects with high (>45 ng/mL) and low (<45 ng/mL) CC16 concentration on day 0. This cut-off level was applied based on our previous analysis from FACTT trial which included 68 patients. Secondary outcomes included ICU-free days, ventilator-free days (VFD), and organ failure-free days.

Table 1. Characteristics and clinical outcomes of ALTA patients.

CHARACTERISTIC	TOTAL COHORT (N = 100)	LOW CC16 (≤45 NG/ML) (N = 47)	HIGH CC16 (>45 NG/ML) (N = 53)	*P*-VALUE
Age	50.7 ± 15.6	47.2 ± 14.4	53.9 ± 16.0	.03
Female sex, %	46	36.2	54.7	.06
APACHE III score	92.3 ± 28.8	76.8 ± 19.9	105.9 ± 4.0	<.001
PaO_2/FiO_2	141.9 ± 59.9	149.2 ± 66.3	135.5 ± 53.5	.2
Patient outcomes				
90-day mortality %	26	8.9	37.7	<.001
Ventilator-free days	19 (0, 23)	21 (11, 24)	15 (0, 23)	.03
ICU-free days	16 (4, 22)	18 (9, 23)	14 (0, 21.5)	.04
Organ failure free days	16 (2.25, 25)	23 (10, 26)	8 (0, 20)	<.001

Abbreviations: APACHE, Acute Physiology, Age and Chronic Health Evaluation; PaO_2, Partial pressure of oxygen; FiO_2, fraction of inspired oxygen.
Continuous data presented as means ± SD or median (interquartile range) as indicated.

The ALTA and FACTT trials are multicenter, prospective, randomized, clinical trials of intubated patients with acute lung injury. The designs of both trials have been described in detail previously.[7,8] Briefly, intubated patients with bilateral lung infiltrates consistent with the presence of pulmonary edema not related to left atrial hypertension, had a ratio partial pressure of oxygen to fraction of inspired oxygen (PaO_2/FiO_2) of 300 or less were included. Patients with acute myocardial infarction, left atrial hypertension, neuromuscular disease, high risk of mortality within 6 months, long term respiratory or hepatic diseases were excluded. Subjects of ALTA were randomized to receive aerosolized albuterol or placebo whereas FACTT patients were assigned to either conservative or liberal fluid management. The study period of ALTA trial was between August 2007 and September 2008. FACTT study enrollment was between June 2000, and October 2005.

The current study is a retrospective biomarker analysis of the ALTA trial. Plasma samples were obtained from the National Heart, Lung, and Blood Institute's (NHLBI) Biological Specimen and Data Repository Information Coordinating Center (BioLINCC). Analyses took place at Augusta University laboratories from May to August 2022. This study was approved by the Augusta University Institutional Review Board (IRB number: 1128838-13).

The estimation of the sample size in the current study was based on our previous binomial proportions for the 90-day mortality outcome (50% vs 7.5% in high vs low CC16 groups, respectively).[6] We calculated that enrolling 102 patients (51 in each group) would provide a statistical power of 95% at an α level of .001 to validate the previous difference in mortality. Due to limited samples obtained from BioLINCC, and unknown CC16 concentrations in samples, we later grouped 53 patients with high CC16 and 47 with low CC16.

Statistical analysis

All statistical analyses were computed in IBM SPSS Statistics Version 27.0 and figures were created with GraphPad Prism. Statistical significance was set at an alpha of 0.05. Patient demographics were evaluated using descriptive statistics. The primary outcome was assessed with Kaplan-Meier curves and a log-rank test. Categorical variables were assessed with Chi-squared analysis. The best cut-off value was assessed using the maximum Youden's index (sensitivity + specificity – 1) as standard. For normal distribution data, mean and standard deviation were reported, whereas median and interquartile ranges were stated for nonnormal distribution data. Analysis of variance and Mann Whitney *U*-test were utilized as parametric and nonparametric tests, respectively to calculate the *P* value.

Results

Patient characteristics

In this cohort of 100 ALTA patients, the average age was 50 years old with a 26% mortality rate. Baseline characteristics can be seen in Table 1. Patients in the high CC16 group were significantly older (47 vs 53; $P = .03$), and had a higher average APACHE III score (76.8 vs 105.9; $P < .001$). Other baseline characteristics were similar between groups.

Patient outcomes

When stratified by our previously defined CC16 cut point of 45 ng/mL, patients in the high CC16 group had significantly higher mortality (37.7% vs 8.9%, $P < .001$) than those with low baseline CC16 (Figure 1A). In addition to increased mortality, patients with high baseline CC16 has significantly fewer VFD (21 vs 15; $P = .03$), organ failure-free days (23 vs 8; $P < .001$), and ICU free days (18 vs 14; $P = .04$). Upon independent area

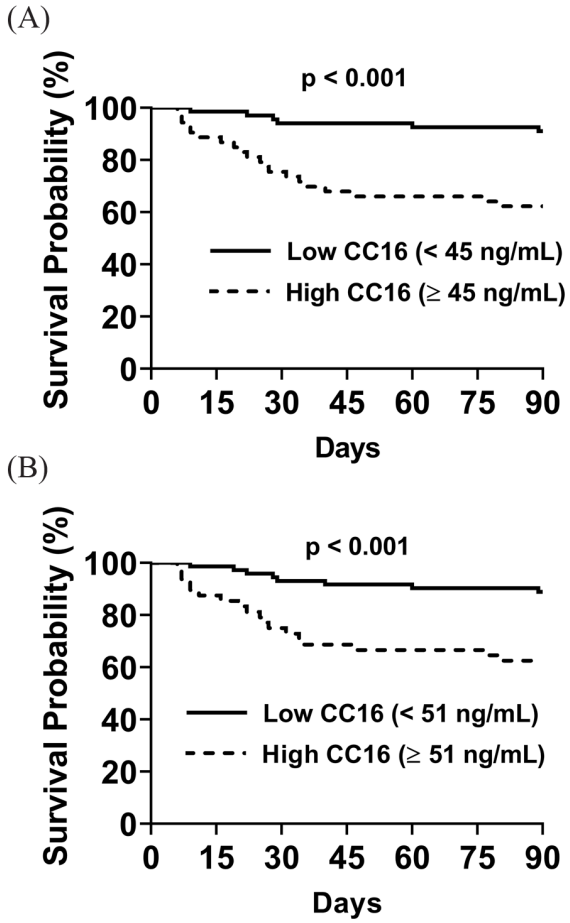

Figure 1. Kaplan-Meier survival curve high vs low CC16 concentration. Kaplan-Meier survival curve for ARDS patients (n = 100) from ALTA trial was censored at 90-days follow-up based on a 45 ng/mL cut-off level (A) and a 51 ng/mL cut-off level (B).

under the receiver operating characteristics (AUROC) analysis of the ALTA cohort CC16 vs mortality, the AUROC was 0.67, whereas the optimal cut point was 48 ng/mL. Lastly, we applied another cut-off level (51 ng/mL) reported by Lin et al which was associated with 28-day mortality in ICU patients with ARDS.[4] Likewise, patients with high CC16 levels (⩾51 ng/mL) had higher 90-day mortality compared to those with low CC16 levels (<51 ng/mL), (37.5% vs 11.11%, $P < .001$) (Figure 1B).

Discussion

This study provides further evidence that high baseline CC16 levels defined as ⩾45 ng/mL are associated with increased mortality in patients with ARDS and may serve as a useful prognostic indicator. Our previous analysis of 68 patients from the FACTT trial demonstrated an optimal CC16 cut point of 45 ng/mL for the prediction of 90-day mortality. In our current study, utilization of this same cut point to stratify patients in an independent cohort demonstrated a large and significant difference in 90-day mortality in an independent cohort. While the independent analysis of the ALTA cohort yielded a lower AUROC (0.67), the optimal stratification level remained similar to our previous findings, validating a cut point near 45 ng/mL. Nevertheless, stratification by this CC16 concentration for ARDS prognosis warrants prospective analysis.

The primary and secondary outcomes are quite similar in our previous and current study including differences in 90-day mortality, VFDs, and ICU-free days. In both studies, patients with high CC16 concentration had higher 90-day mortality. However, worse status in other clinical outcomes like VFD and ICU-free days can be seen only in ALTA but not FACTT patients. This could be explained by the age of ARDS samples in FACTT compared to those in ALTA (7 years older), as well as the difference in sample size (100 vs 68 patients in ALTA and FACTT, respectively). Thus, broader prospective validation with a larger sample size is needed to draw inferential conclusions and provide generalizability.

Our findings are comparable to what has been found in previous observational studies of CC16 in ARDS.[3,4] Lin et al reported that CC16 level was associated with the severity of ARDS assessed by ICU length of stay and PaO_2/FiO_2 ratio. They also reported higher levels of CC16 in non-survivors than in survivors. Moreover, Lesur et al found that high baseline CC16 concentration was associated with worse clinical outcomes in ARDS patients including death, ventilator-free days, and multiple organ failure.[9] In the present study, patients with elevated CC16 had similar worse outcomes. Taken together, these findings support the potential of CC16 as a prognostic biomarker for ARDS.[3-5,10]

Club cells, which are mainly located in the bronchial tubes are the primary source of the CC16 protein.[11] These cells are reported to have roles in the repair of the damaged epithelium as they migrate and replace injured alveoli during lung injury remodeling.[12-15] Due to the increased vascular permeability of the alveoli in ARDS, CC16 protein secreted from Club cells may easily diffuse into circulation. Furthermore, diffuse alveolar damage (DAD), a histological feature for the acute stage of ARDS, is associated with a higher mortality rate.[16] Therefore, elevated levels of CC16 in systemic circulation may indicate more severe damage in the alveolar epithelium and predict a poor prognosis of ARDS.

Laboratory-based blood markers for prognostication and phenotype-based therapies have been proposed for the management of ARDS.[17-19] However, its application has mainly focused on inflammatory markers so far.[17,20,21] Incorporating lung-specific biomarkers into predictive models may provide a better understanding of the pathophysiologic alterations of phenotype-based prognostication and response to therapy.[21] In our studies, the higher CC16 subgroup appeared to have worse outcomes as well. Therefore, including CC16 in the biomarker panels of ARDS sub-phenotypes is worth further validation.

Early detection and intervention are critical to improving outcomes in patients with ARDS, and the time course of CC16 plasma concentration supports its use as an early marker. CC16 appears to be rapidly elevated in circulation after alveolar injury. Wutzler et al reported that admission concentration of CC16 in sera of patients with multiple injuries more accurately identified those with lung injury than others suggesting the importance of early measurement of circulating CC16 from trauma patients at risk of ARDS.[22] Same group of researchers showed that initial elevation of circulating CC16 within 24 hours period was associated with severe lung involvement in trauma patients.[23] In our studies, day 0 or day 1 CC16 predicted poor prognosis and death within 90 days. Taken together, CC16 may constitute an early circulating prognostic biomarker for lung injury and a promising predictor for ARDS-related mortality.

In addition to CC16's potential to serve as a predictive biomarker, CC16 may also have therapeutic roles. CC16 has demonstrated a beneficial effect against the pulmonary inflammatory response by modulation of the activities of phospholipases A2, interferon-γ, and tumor necrosis factor-α (TNF-α) raising a possibility to serve as a potential therapy for lung diseases.[24] Therefore, several studies aimed to examine the role of CC16 using a neutralizing antibody. In a murine model of sepsis-induced lung injury as described by blunt chest trauma with subsequent cecal ligation and puncture, CC16 neutralization caused an increased neutrophilic infiltration and pro-inflammatory CXCL1 levels.[25,26] Another study utilizing the same experimental method showed increased pro-inflammatory cytokines, immune cells infiltration and more severe pulmonary damage after CC16 neutralization.[27] Taken together, endogenous CC16 appeared to be an anti-inflammatory pulmonary protein with protective roles in ALI.

This study has several key limitations including the retrospective nature and limited sample size. We did not reach our intended sample size (n = 51 in each group), therefore the risk of type II error cannot be ruled out; however, since we observed a difference in the primary outcome this is less of a concern. Another limitation is the difference in AUROC values between ALTA (0.67) and FACTT (0.78) cohorts. The apparent difference between the AUROC could be due to several variables including the age of FACTT samples, the variable sample size, and the different timing of sample collection from each cohort. Still, our previously reported values are consistent with the available literature.

Conclusion

In this study, we demonstrated that stratification of an independent cohort of ARDS patients by our previously reported prognostic cut point yielded similar results. Patients in the high CC16 group had significantly higher mortality than those with low baseline CC16. CC16 warrants further prospective validation as a prognostic biomarker among ARDS patients.

Declarations

Ethics approval and consent to participate

Public-use data and plasma specimens were collected from the ALTA trial and provided via BioLINCC. All research procedures in ALTA trial were approved by the institutional review boards and informed consent in writing was obtained from enrolled patients or surrogates at each participating hospital. Plasma samples were measured at Augusta University laboratories and this study was approved by the Augusta University Institutional Review Board (IRB number: 1128838-13).

Consent for publication

This study was approved by the Augusta University Institutional Review Board.

Author contributions

Sultan Almuntashiri: Conceptualization; Data curation; Formal analysis; Writing – original draft. Aaron Chase: Formal analysis; Investigation; Validation; Writing – review & editing. Andrea Sikora: Conceptualization; Formal analysis; Investigation; Writing – review & editing. Duo Zhang: Conceptualization; Funding acquisition; Investigation; Supervision; Writing – review & editing.

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Availability of data and materials

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

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