RESEARCH ARTICLE

WILEY

# Diagnostic and prognostic values of Club cell protein 16 (CC16) in critical care patients with acute respiratory distress syndrome

Jinle Lin[1] | Wenwu Zhang[1] iD | Lijun Wang[2] | Fang Tian[2]

[1]Department of Emergency and Critical Care Medicine, Baoan Hospital, Nanfang Medical University, Shenzhen, Guangdong, China

[2]Department of Critical Care Medicine, Baoan Hospital, Nanfang Medical University, Shenzhen, Guangdong, China

**Correspondence**
Wenwu Zhang, Department of Emergency and Critical Care Medicine, Baoan Hospital, Nanfang Medical University, Baoan, Shenzhen, Guangdong, China.
Email:eccm_bah@foxmail.com

**Background:** Acute respiratory distress syndrome (ARDS) is a critical condition characterized by bilateral pulmonary infiltrates and severe hypoxemia. This study aimed to evaluate the diagnostic and prognostic values of Club cell protein 16 (CC16) in critical care patients with ARDS.

**Methods:** In this retrospective observational study, 83 patients with ARDS and 129 non-ARDS patients on ICU admission were enrolled. The differences in serum CC16 and other laboratory indicators between two groups were analyzed. The sensitivity, specificity, positive and negative predictive values, and accuracy of CC16 as a diagnostic marker on ICU admission were determined by receiver operating characteristic (ROC) curve analysis. The correlation between serum CC16 levels and the severity of ARDS as quantified by $PaO_2/FiO_2$ ratio were further assessed. CC16 levels were compared between survivors and non-survivors. The relationships between CC16 levels and duration of ICU and hospitalization were evaluated.

**Results:** The serum CC16 levels in ARDS patients were significantly higher than that in non-ARDS patients (54.44±19.62 vs 24.13±12.32 ng/mL, *P*=.001). ROC analysis showed that the sensitivity, specificity, positive predictive value, and negative predictive value were 90.4%, 79.8%, 74.2%, and 92.8%, respectively, when the cut-off value was set at 33.3 ng/mL. CC16 levels were correlated with the severity of ARDS. The serum CC16 levels were significantly greater in non-survivors than in survivors from the ARDS group. CC16 levels were associated with ICU stay but not hospital stay.

**Conclusions:** CC16 may serve as a diagnostic and stratification marker for ARDS. However, it provided limited prognostic information for ARDS.

KEYWORDS
acute respiratory distress syndrome, Berlin definition, biomarkers, Club cell protein 16

## 1 | INTRODUCTION

Acute respiratory distress syndrome (ARDS), characterized by permeability pulmonary edema and refractory hypoxemia, has an enormous effect on both morbidity and mortality.[1–3] The Berlin definition is widely accepted for diagnosing ARDS, and the diagnosis is predominantly based on oxygenation index and chest X-ray.[4] However, there are no definite laboratory indicators serving the diagnosis and prognosis of ARDS.

Biomarkers, which are often objectively measured and not biased by personal interpretation, may facilitate the fast diagnosis

and risk stratification of ARDS. Although various biomarkers, such as surfactant protein-D (SP-D), soluble receptor for advanced glycation end-products (sRAGE), and Krebs von den Lungen (KL-6), have been investigated for possible use in ARDS diagnosis, their clinical utility is limited due to the lack of discriminatory power.[5] Club cell protein (CC16), an anti-inflammatory protein mainly produced and secreted by the Club cells in the distal respiratory or terminal bronchioles, has been proposed as a biomarker for lung epithelial injury.[6] Considering the large blood-tissue exchange surface area of lung tissues, serum CC16 is almost solely derived from the respiratory tract and is, therefore, considered lung-specific. Increased circulating CC16 levels have been observed in patients with pulmonary injuries caused by inhaled ozone, chlorine, and LPS.[7-9] It has been suggested as a disease marker for pulmonary sarcoidosis, chronic obstructive pulmonary disease, and severe chest trauma.[10-12] However, whether the CC16 levels can be used in ARDS diagnosis, classification, and outcome prediction remains unclear, although a few clinical studies have indicated that circulating CC16 levels are associated with the development of ARDS.[13]

In this study, we investigated the serum levels of CC16 in patients with and without ARDS in order to test our hypothesis that serum CC16 may help distinguish and stratify ARDS patients, correlated with the prognostic parameters such as survival and length of hospital stay. Our results have clinical implications for ARDS management.

## 2 | METHODS

### 2.1 | Patients and study design

This study was reviewed and approved by the Ethics Committee of Baoan Hospital of Nanfang Medical University, and a written informed consent was obtained from each of the participants or their legal surrogates.

The study retrospectively analyzed 212 patients who were consecutively admitted into our comprehensive intensive care unit (ICU) between March 1, 2013, and December 31, 2014. The diagnosis of ARDS was made within 24 hours after ICU admission, according to
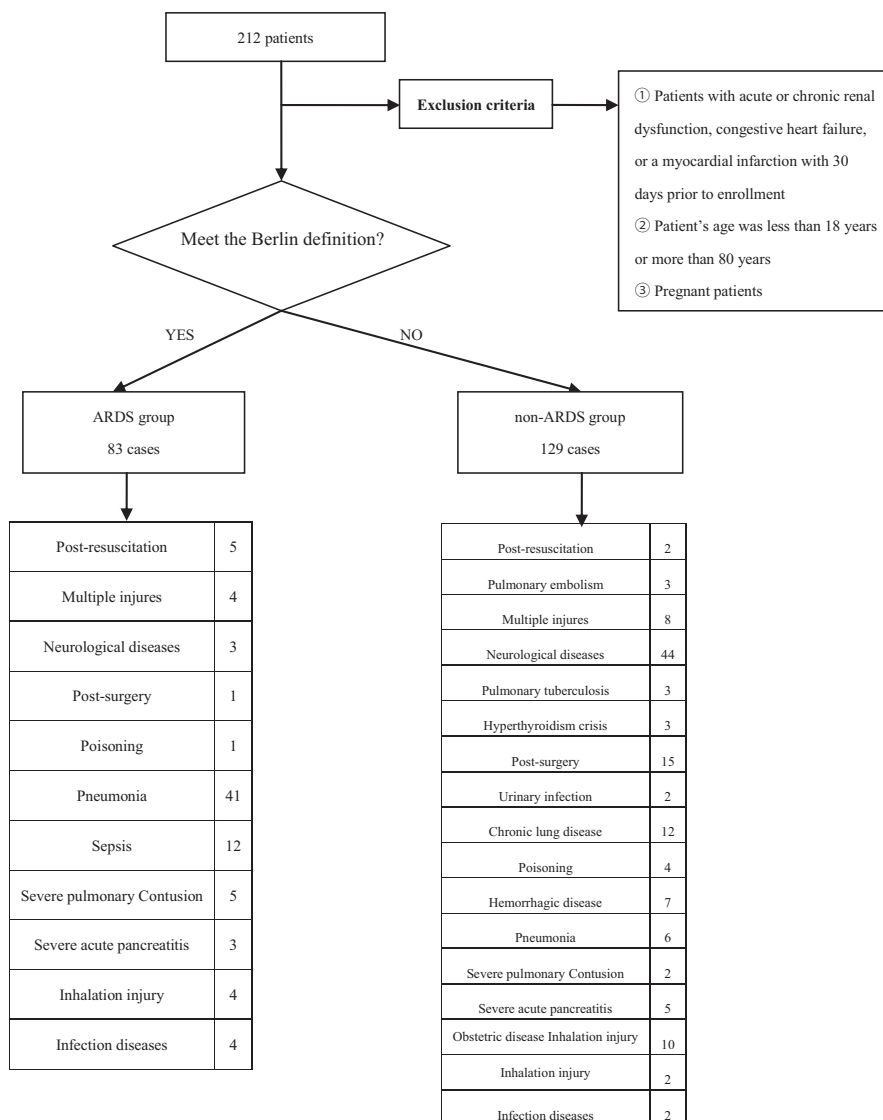


**FIGURE 1** A flow chart of the study design

**TABLE 1** Characteristics of ARDS and non-ARDS patients

| Index | ARDS | Non-ARDS | P |
|---|---|---|---|
| Baseline characteristics | | | |
| Age (yrs) | 54.25±20.32 | 49.0±19.5 | .176 |
| Male (%) | 63.9% | 55.8% | .245 |
| WBC (×10$^9$/L) | 12.63±6.65 | 12.20±5.70 | .398 |
| CRP (mg/L) | 108.03±76.82 | 67.52±66.10 | .001* |
| NT-proBNP (pg/mL) | 489.67±942.44 | 310.43±572.41 | .096 |
| Albumin (g/L) | 28.56±7.26 | 32.01±8.16 | .002* |
| Scr (μmol/L) | 87.05±42.34 | 67.32±22.88 | .001* |
| Serum CC16 (ng/mL) | 54.44±19.62 | 24.13±12.32 | .001* |
| Pre-admission intubations (%) | 45.8% | 50.4% | .513 |
| APACHE II score | 21.0±6.3 | 19.6±4.8 | .090 |
| Patient outcomes | | | |
| Duration of MV (days) | 7.37±6.57 | 5.64±10.88 | .194 |
| Length of ICU stay (days) | 11.63±11.32 | 11.29±14.95 | .863 |
| Length of hospital stay (days) | 25.90±39.70 | 23.65±24.89 | .612 |
| 7-day mortality | 28.9% | 11.6% | .002* |
| 28-day mortality | 42.2% | 16.3% | .001* |

WBC, white blood cell count; CPR, C-creative protein; NT-proBNP, the N-terminal of the prohormone brain natriuretic peptide; Scr, serum creatinine concentration; CC16, Club cell protein 16; APACHE II: Acute Physiology and Chronic Health Evaluation II; MV, mechanical ventilation; ICU, intensive care unit.

Values are presented as mean±SD. *$P<.05$.

the Berlin definition: (i) acute, meaning onset within 1 week; (ii) bilateral opacities consistent with pulmonary edema as detected by CT or chest radiography; (iii) $PaO_2/FiO_2$ ratio <300 mm Hg with a minimum of 5 cmH$_2$O PEEP (or CPAP); and (iv) must not be fully explained by cardiac failure or fluid overload. ARDS patients were then divided into three categories based on the degree of hypoxemia: mild ($PaO_2/FiO_2$<300 mm Hg, n=20), moderate ($PaO_2/FiO_2$<200 mm Hg, n=26), and severe ($PaO_2/FiO_2$<100 mm Hg, n=11) (Figure 1). The $PaO_2/FiO_2$ ratio was chosen as the worst value within 2 hours on ICU admission. All patients meeting the above ARDS criteria were included in this study. The exclusion criteria were as follows: (i) patients with acute or chronic renal dysfunction, congestive heart failure, or a myocardial infarction within 30 days prior to enrollment; (ii) patient's age was less than 18 years or more than 80 years; and (iii) pregnant patients. All enrolled patients were followed up for 28 days. A radiologist, blinded to the clinical phenotype and CC16 measurement, reviewed radiographs.

## 2.2 | Laboratory examination

Blood samples were drawn from the radial artery within 2 hours of ICU admission; the analyses included white blood cell count (WBC), C-reactive protein (CRP), the N-terminal of the prohormone brain natriuretic peptide (NT-proBNP), albumin, and serum creatinine (Scr).
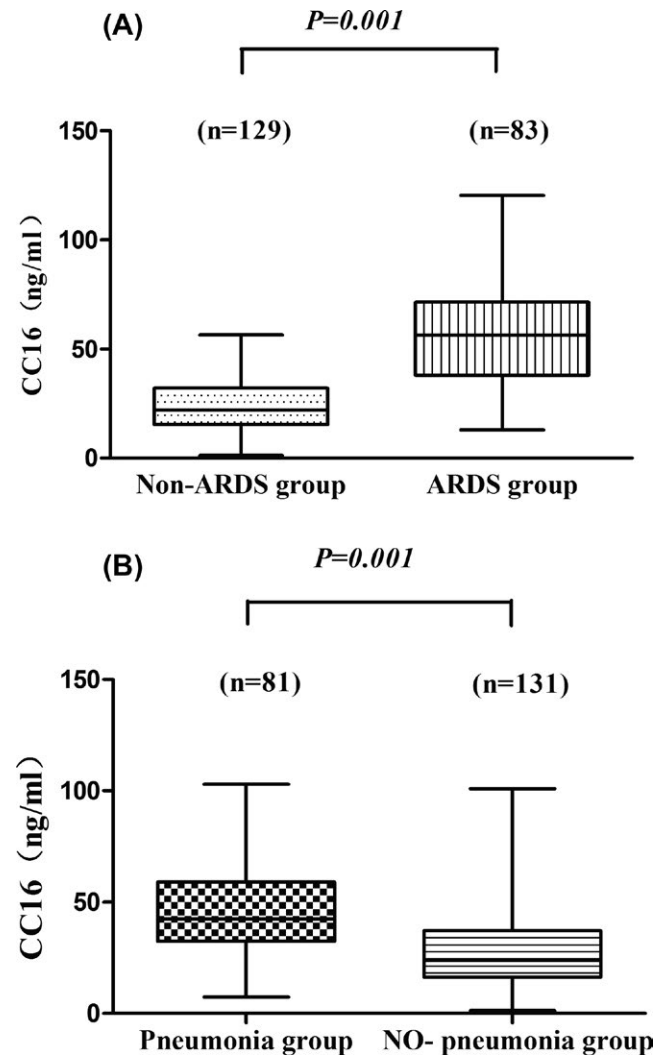


**FIGURE 2** Comparison of serum CC16 levels among different groups. A, Non-ARDS group vs ARDS group. B, Patients with pneumonia vs patients without pneumonia

For the measurement of CC16, the blood samples were immediately centrifuged at 1580 $g$ for 10 minutes, and the serum was stored at −60°C until analysis. CC16 concentration was determined using ELISA kits (R&D Systems, Minneapolis, MN, USA) following the manufacturer's instructions. Each assay was performed in duplicate. The laboratory staff who performed the analyses were blinded to clinical phenotypes.

## 2.3 | Data processing and statistical analysis

The $PaO_2/FiO_2$ ratio and Acute Physiology and Chronic Health Evaluation II (APACHE II) score were calculated upon patient admission. Clinical data including age, gender, $PaO_2/FiO_2$ ratio, pre-admission intubation rate, APACHE II score, duration of MV (mechanical ventilation), length of ICU stay, 7-day and 28-day mortality, and blood test results were compiled in a spread-sheet format (Microsoft Office Excel 2003; Microsoft Corp, Seattle, WA) for subsequent analysis.

| Index | Regression coefficient | Wald | P | OR (95%CI) |
|---|---|---|---|---|
| Age (yrs) | 0.013 | 3.471 | .062 | 1.013 (0.999, 1.028) |
| Male (%) | −0.335 | 1.346 | .246 | 0.715 (0.406, 1.260) |
| WBC (×10$^9$/L) | 0.012 | 0.255 | .614 | 1.012 (0.967,1.058) |
| CRP (mg/L) | 0.008 | 13.45 | .001[*] | 1.008 (1.004,1.012) |
| NT-proBNP (ng/mL) | 0.001 | 2.500 | .114 | 1.000 (1.000,1.001) |
| Albumin (g/L) | −0.057 | 9.040 | .003[*] | 0.945 (0.911,0.980) |
| Scr (μmol/L) | 0.020 | 15.01 | .001[*] | 1.020 (1.010,1.030) |
| Serum CC16 (ng/mL) | 0.122 | 54.22 | .001[*] | 1.130 (1.094, 1.167) |
| Pre-admission intubations (%) | −0.062 | 0.428 | .513 | 0.940 (0.782,1.131) |
| APACHE II score | 0.147 | 3.123 | .075 | 1.136 (1.025,1.258) |

WBC, white blood cell count; CPR, C-creative protein; NT-proBNP, the N-terminal of the prohormone brain natriuretic peptide; Scr, serum creatinine concentration; CC16, Club cell protein 16; APACHE II: Acute Physiology and Chronic Health Evaluation II; MV, mechanical ventilation; ICU, intensive care unit. *P<.05.

Measurements were reported as mean±standard deviation (SD) or median (interquartile range). Student's *t* test or Mann-Whitney *U* test was used for comparisons between ARDS and non-ARDS groups, depending on whether or not the data demonstrated normal distribution. Differences in the concentration of serum CC16 among different groups (mild, moderate, and severe ARDS and non-ARDS) were evaluated with a one-way ANOVA. Variables with categorical data were compared using the $X^2$ or Kruskal-Wallis test. Logistic regression was used to perform multivariate analysis. Receiver operating characteristic (ROC) curves were analyzed to assess the optimal cut-off values of the influencing factors. Sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV) were calculated for the chosen cut-off values. Linear correlations between CC16 levels, $PaO_2/FiO_2$ ratios, and length of hospitalization were calculated using the Pearson correlation coefficient analysis. Statistical analyses were performed using the SPSS package (version 20.0; SPSS for Windows, Armonk, NY). *P*<.05 was considered statistically significant.

## 3 | RESULTS

Eighty-three patients with ARDS and 129 patients without ARDS were recruited into the reported study. There were no significant differences in age, gender, pre-admission intubation rate, APACHE II score, duration of MV, length of ICU stay, and blood test results including WBC and NT-proBNP between the two groups (Table 1).

The mean CC16 concentration in the ARDS group was significantly higher than that in the non-ARDS group (54.44±19.62 vs 24.13±12.32 ng/mL, *P*=.001) (Figure 2A). The differences in CRP, albumin, and Scr between the two groups were also statistically significant (all *P*<.05; Table 1). In addition, 7- and 28-day mortality for patients with ARDS was significantly greater than that in patients without ARDS.

The average levels of CC16 in patients with pneumonia (n=81) and without pneumonia (n=132) were 45.65ng/mL and 30.04ng/mL, respectively (*P*=.001) (Figure 2B). Univariate logistic regression analysis showed that factors in the diagnosis of ARDS included CRP, albumin, Scr, and serum CC16 (Table 2).

Multivariate logistic regression analysis including CRP, albumin, Scr, and serum CC16 as variables showed that CC16 and CRP were independent factors that could be used in the diagnosis of ARDS (Table 3).

ROC analysis of CC16 as a diagnostic marker for ARDS upon patient admission revealed an area under the curve (AUC) of 0.911 (95% confidence interval [CI]: 0.861-0.947; *P*<.001) (Figure 3). Taking the maximum Youden's index (sensibility+specificity − 1) as standard, the optimal cut-off value was assessed. When the cut-off value was set at 33.3 ng/mL, CC16 had a sensitivity of 90.4%, specificity of 79.8%, PPV of 74.2%, and NPV of 92.8% (Figure 3). However, AUC for CRP was 0.648 with a lower sensitivity of 54.4% and specificity of 73.2%.

The mean CC16 level in the severe ARDS group was significantly greater than that in the mild or moderate ARDS groups (64.73±14.42 ng/mL vs 57.35±19.33 ng/mL, *P*=.007; 64.73±14.42 ng/mL vs 48.17±19.81 ng/mL, *P*=.041). The difference in CC16 levels between

| Index | Regression coefficient | Wald | P | OR (95%CI) |
|---|---|---|---|---|
| CRP (mg/L) | 0.007 | 4.993 | .025[*] | 1.007 (1.001, 1.014) |
| Albumin (g/L) | −0.012 | 0.146 | .703 | 0.988 (0.931, 1.049) |
| Scr (μmol/L) | 0.009 | 1.702 | .192 | 0.995 (0.996, 1.023) |
| Serum CC16 (ng/mL) | 0.116 | 43.799 | .001[*] | 1.096 (1.085, 1.162) |

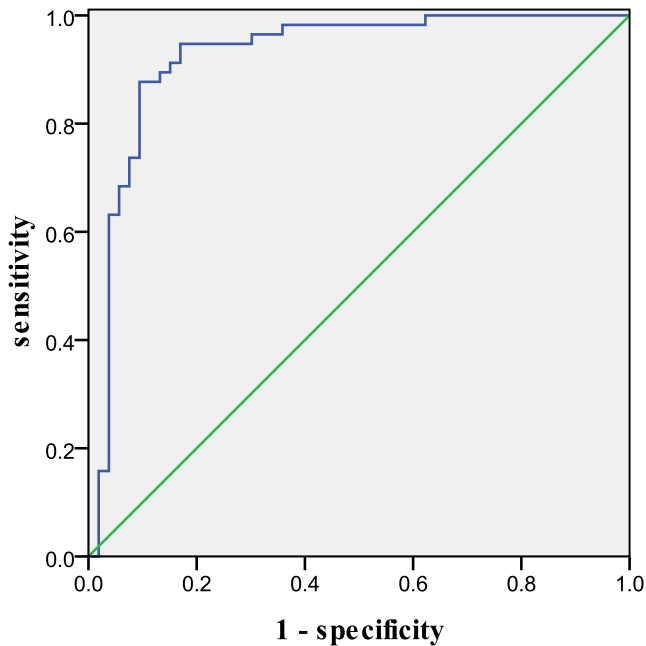CPR, C-creative protein; Scr, serum creatinine concentration; CC16, Club cell protein 16; *P<.05.

**FIGURE 3** ROC curve of serum CC16 values upon patient admission



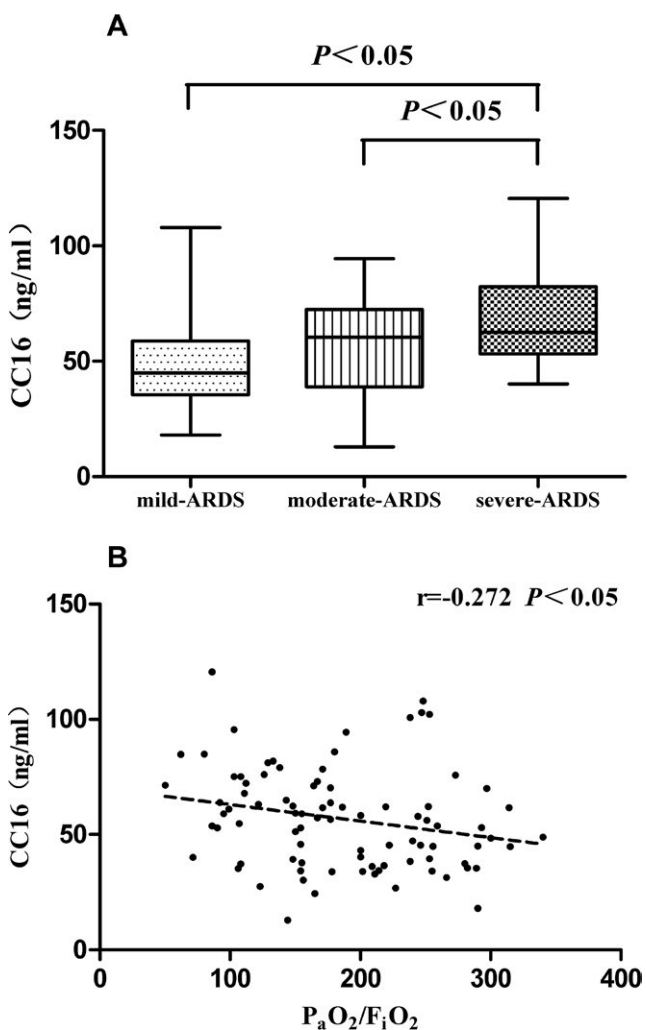**FIGURE 4** Correlation of serum CC16 and $PaO_2/FiO_2$ ratio in ARDS patients upon admission

mild and moderate ARDS groups was not statistically significant (Figure 4A).

There was a significant negative correlation between serum CC16 concentration and $PaO_2/FiO_2$ ratio in ARDS patients ($r=-.272$, $P<.05$; Figure 4B). In the non-ARDS group, the correlation between serum CC16 concentration and $PaO_2/FiO_2$ ratio was not significant (data not shown).

There was a significant difference in serum CC16 levels between survivors and non-survivors from the ARDS group at 7 days and 28 days (Figure 5). A close positive correlation was found between the serum CC16 levels and length of ICU stay among ARDS patients ($r=.159$, $P<.05$). However, similar association was not presented between the serum CC16 levels and the stay of hospital ($r=.098$, $P>.05$; Figure 6).

## 4 | DISCUSSION

In this study, elevated circulating CC16 levels identified ARDS and demonstrated a strong correlation with the severity of the disease as assessed by $PaO_2/FiO_2$ ratio. Our analyses showed that CC16 was an independent factor that facilitated diagnosis of ARDS. In addition, CC16 levels differed significantly between survivors and non-survivors in ARDS patients and were correlated with ICU stay, but not hospital stay.

Increases in serum or plasma CC16 levels have been documented in studies of lung injuries caused by multiple etiologies, including systemic sclerosis, mechanical ventilation, air pollution, pulmonary sarcoidosis, and lung transplantation.[10,14–17] Our results demonstrating that CC16 rose significantly in ARDS patients were consistent with these studies. ARDS patients in the present study had more than double the median values of CC16 when compared with non-ARDS patients. While previous studies have demonstrated a relatively stable median CC16 value in normal controls (5-7 ng/mL),[12,13] the median CC16 value of non-ARDS patients in this study appeared to be higher (24.13±12.32 ng/mL). Some cases from the non-ARDS patient group had developed pulmonary diseases such as pneumonia, which may have caused an elevated production of CC16.[18] The compromised renal function indicated by an increase of Scr in some non-ARDS patients may be another reason accounting for the difference in the CC16 levels since a decreased glomerular filtration rate retards the clearance of CC16.[19]

Our results supported the notion that changes in circulating CC16 levels associated with chronic lung diseases were inconsistent with changes that occurred due to acute lung injury. Previous studies indicate that circulating CC16 levels are decreased in lung injures caused by agents like tobacco or hexavalent chromium.[20,21] Chronic exposure to toxic agents causes a decline in the overall Clara cells located in terminal and respiratory bronchioles, leading to a relatively low production level of circulating CC16. Meanwhile, CC16 levels may vary due to differences in sample collection, measurement time points, and methods used in different studies. For example, a previous study of chemical-induced lung injury in rats has reported decreased serum CC16 levels after an initial transient
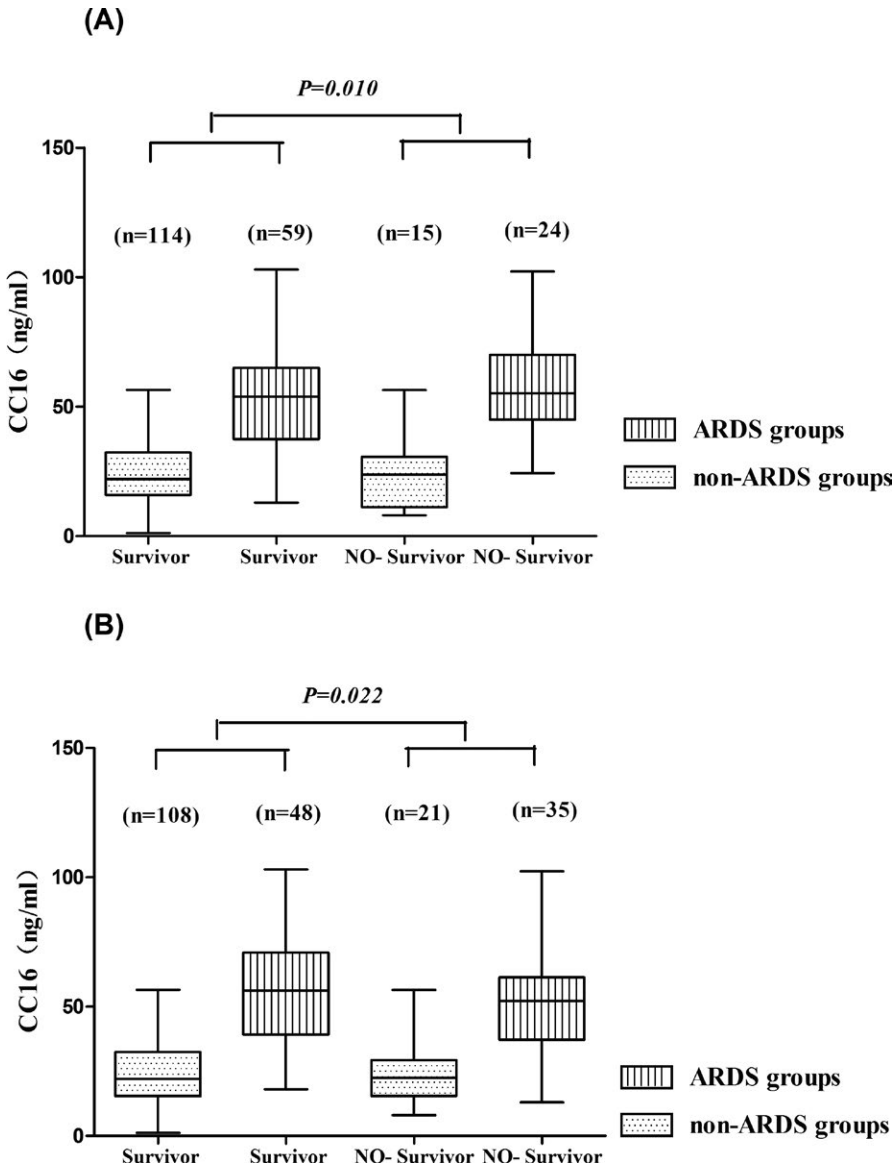
**(A)**



**(B)**



**FIGURE 5** Comparison of serum CC16 levels between survivors and non-survivors from ARDS group (A, 7 days; B, 28 days)

increase compared to saline solution-treated control animals.[20] Some studies have also revealed that CC16 levels in bronchoalveolar fluid (BALF) differ from blood CC16 levels. Following lung damage induced by mechanical ventilation (MV), the CC16 content is decreased in BALF but increased in the bloodstream.[15] The differing CC16 level changes are believed to be due to increased transepithelial leakage of CC16 along with dilution of BALF during collection. A comparable standard of measurement is, therefore, necessary for future studies.

This study confirms that CC16 could be applied as an efficient diagnostic marker for ARDS. Our results showed that CC16 is an independent factor that can facilitate diagnosis. The CC16 values of above 33.3 ng/mL statistically discriminated differentiates between ARDS and non-ARDS patients, demonstrating a relatively high diagnostic accuracy. Although we did not compare the diagnostic value of CC16 with other biomarkers in this study, previous studies have demonstrated that CC16 had greater diagnostic capacity than surfactant protein-D (SP-D), Krebs von den Lungen-6 (KL-6), and soluble receptor for

advanced glycation end-products (sRAGE) for ARDS.[13] A combination of CC16, SP-D, and KL-6 could provide enhanced efficacy in lung disease screening and diagnosis.[14] In addition, an increase in CC16 has been demonstrated 2 days before clinical diagnosis of ARDS and an increase in serum CC16 of 30% or more has a sensitivity of 90% and specificity of 92% for diagnosis of ARDS.[22]

Based on the Berlin definition, ARDS was classified during this study as mild, moderate, and severe, according to the $PaO_2/FiO_2$ ratio. We observed a significant difference in serum CC16 values between the severe and moderate/mild ARDS patients. Serum CC16 levels were negatively correlated with $PaO_2/FiO_2$ value, indicating a close relationship between CC16 levels and the disease severity. To our best knowledge, this was the first study showing a correlation of CC16 with the severity of ARDS. Similar correlations were observed between CC16 and indicators of lung function in chromium-induced lung injury, diffusion capacity, and total lung capacity in pulmonary sarcoidosis, and contused lung parenchyma tissue volume in multiple injuries.[10,12,22] In a recent study of asbestos-induced lung disorder,
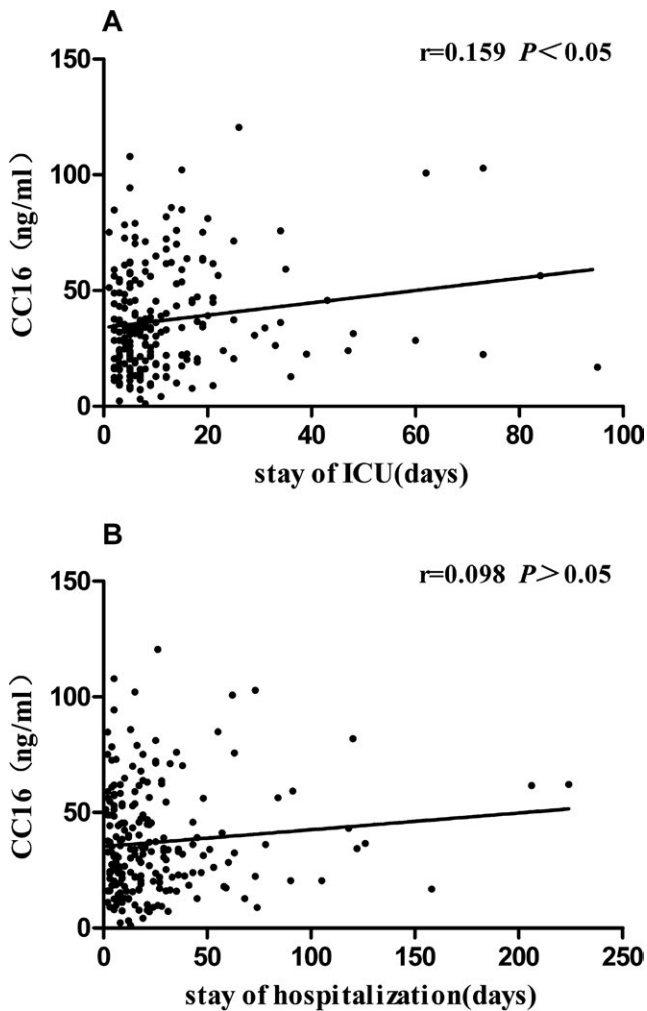
**FIGURE 6** Correlation of serum CC16 with ICU and hospital stay

however, no association was found between the levels of CC16 in serum or BALF and the severity of lung impairment as assessed by chest X-ray.[23] A relatively small cohort and late detection in that study may explain the difference.

Controversies exist concerning the predictive value of CC16 on patient admission. Our findings demonstrated that there existed a significant difference in CC16 levels between survivors and non-survivors among these ARDS patients, indicating that the CC16 concentration may have predictive value in patient survival. In addition, there was a close correlation between the CC16 levels and the durations of ICU stay among ARDS patients. However, no similar correlation was found between CC16 and stay of hospitalization. Kropskiet al. have tested the prognostic utility of CC16 in patients with non–trauma-related ARDS compared with a control group of patients with acute cardiogenic pulmonary edema and demonstrated that neither plasma nor edema fluid CC16 levels facilitate mortality prediction, the number of days of unassisted ventilation, or ICU length of stay.[24] However, a contrary conclusion was made in another clinical study where serum CC16 was indicated as one of the predictors of mortality in ARDS patients.[25] We speculate that different study populations result in this discrepancy in results.

A major strength of this study was the application of a blood biomarker for diagnosis of ARDS, which is often difficult to distinguish using clinical and radiographic criteria. Furthermore, we demonstrated that the serum CC16 level correlated closely with the severity of ARDS as assessed by $PaO_2/FiO_2$ ratio. However, the current study had some limitations. First, the current study design may have the potential for selection bias. The discrepancy of basic diseases between the ARDS and non-ARDS group might limit the clinical application of this study. In addition, the mortality of ARDS group in this study seems to be higher than other reported studies.[1-3] This is probably due to the heterogeneity of different study populations. Second, the overall number of subjects was modest, limiting the statistical power of the study as well as our ability to control statistically for potential confounding factors in multivariate analysis. Third, we only monitored CC16 levels upon patient admission. The clinical course of CC16 during the hospitalization was not reported. Finally, we did not evaluate combined biomarkers for diagnosing ARDS. Therefore, it is necessary to evaluate the diagnostic and prognostic values of CC16 levels in large-scale, prospective clinical studies. In addition, the fact that blood test was not totally non-invasive and time-consuming might limit the clinical application.

In conclusion, serum CC16 level reflects the severity of ARDS and could help differentiate between different categories of ARDS. However, it provides limited prognostic values for ARDS.

## REFERENCES

1. Brun-Buisson C, Minelli C, Bertolini G, et al. Epidemiology and outcome of acute lung injury in European intensive care units. Results from the ALIVE study. *Intensive Care Med*. 2004;30:51-61.
2. Lewandowski K, Lewandowski M. Epidemiology of ARDS. *Minerva Anestesiol*. 2006;72:473-477.
3. Villar J, Blanco J, Añón JM, et al. The ALIEN study: incidence and outcome of acute respiratory distress syndrome in the era of lung protective ventilation. *Intensive Care Med*. 2011;37:1932-1941.
4. ARDS Definition Task Force, Ranieri VM, Rubenfeld GD, et al. Acute respiratory distress syndrome: the Berlin Definition. *JAMA*. 2012;307:2526-2533.
5. Binnie A, Tsang JL, dos Santos CC. Biomarkers in acute respiratory distress syndrome. *Curr Opin Crit Care*. 2014;20:47-55.
6. Dushianthan A, Goss V, Cusack R, Grocott MP, Postle AD. Altered molecular specificity of surfactant phosphatidycholine synthesis in patients with acute respiratory distress syndrome. *Respir Res*. 2014;15:128.
7. Blomberg A, Mudway I, Svensson M, et al. Clara cell protein as a biomarker for ozone-induced lung injury in humans. *Eur Respir J*. 2003;22:883-888.
8. Bonetto G, Corradi M, Carraro S, et al. Longitudinal monitoring of lung injury in children after acute chlorine exposure in a swimming pool. *Am J Respir Crit Care Med*. 2006;174:545-549.
9. Michel O, Murdoch R, Bernard A. Inhaled LPS induces blood release of Clara cell specific protein (CC16) in human beings. *J Allergy Clin Immunol*. 2005;115:1143-1147.

10. Janssen R, Sato H, Grutters JC, et al. Study of Clara cell 16, KL-6, and surfactant protein-D in serum as disease markers in pulmonary sarcoidosis. *Chest*. 2003;124:2119-2125.

11. Park HY, Churg A, Wright JL, et al. Club cell protein 16 and disease progression in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2013;188:1413-1419.

12. Wutzler S, Lehnert T, Laurer H, et al. Circulating levels of Clara cell protein 16 but not surfactant protein D identify and quantify lung damage in patients with multiple injuries. *J Trauma*. 2011;71:E31-E36.

13. Determann RM, Millo JL, Waddy S, Lutter R, Garrard CS, Schultz MJ. Plasma CC16 levels are associated with development of ALI/ARDS in patients with ventilator-associated pneumonia: a retrospective observational study. *BMC Pulm Med*. 2009;9:49.

14. Hasegawa M, Fujimoto M, Hamaguchi Y, et al. Use of serum clara cell 16-kDa (CC16) levels as a potential indicator of active pulmonary fibrosis in systemic sclerosis. *J Rheumatol*. 2011;38:877-884.

15. Lesur O, Hermans C, Chalifour JF, et al. Mechanical ventilation-induced pneumoprotein CC-16 vascular transfer in rats: effect of KGF pretreatment. *Am J Physiol Lung Cell Mol Physiol*. 2003;284: L410-L419.

16. Provost EB, Chaumont A, Kicinski M, et al. Serum levels of club cell secretory protein (Clara) and short- and long-term exposure to particulate air pollution in adolescents. *Environ Int*. 2014;68:66-70.

17. Shah RJ, Wickersham N, Lederer DJ, et al. Preoperative plasma club (clara) cell secretory protein levels are associated with primary graft dysfunction after lung transplantation. *Am J Transplant*. 2014;14:446-452.

18. Laucho-Contreras ME, Polverino F, Gupta K, et al. Protective role for club cell secretory protein-16 (CC16) in the development of COPD. *Eur Respir J*. 2015;45:1544-1556.

19. Hantson P, Bernard A, Hermans C. Kinetics and determinants of the changes of CC16, a lung secretory protein in a rat model of toxic lung injury. *Clin Toxicol (Phila)*. 2008;46:230-238.

20. Li P, Li Y, Zhang J, et al. Biomarkers for lung epithelium injury in occupational hexavalent chromium-exposed workers. *J Occup Environ Med*. 2015;57:e45-e50.

21. Robin M, Dong P, Hermans C, Bernard A, Bersten AD, Doyle IR. Serum levels of CC16, SP-A and SP-B reflect tobacco-smoke exposure in asymptomatic subjects. *Eur Respir J*. 2002;20:1152-1161.

22. Wutzler S, Backhaus L, Henrich D, et al. Clara cell protein 16: A biomarker for detecting secondary respiratory complications in patients with multiple injuries. *J Trauma Acute Care Surg*. 2012;73:838-842.

23. Petrek M, Hermans C, Kolek V, Fialova J, Bernard A. Clara cell protein (CC16) in serum and bronchoalveolar lavage fluid of subjects exposed to asbestos. *Biomarkers*. 2002;7:58-67.

24. Kropski JA, Fremont RD, Calfee CS, Ware LB. Clara cell protein (CC16), a marker of lung epithelial injury, is decreased in plasma and pulmonary edema fluid from patients with acute lung injury. *Chest*. 2009;135:1440-1447.

25. Lesur O, Langevin S, Berthiaume Y, et al. Outcome value of Clara cell protein in serum of patients with acute respiratory distress syndrome. *Intensive Care Med*. 2006;32:1167-1174.