Renal dysfunction decreases specificity and overturns cutoff value of serum CC16 while prohibiting 7-day mortality prediction in diagnosing acute respiratory distress syndrome in intensive care unit.

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Research article

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Abstract

Background: A contradictory tendency between occurrence of acute respiratory distress syndrome (ARDS) and serum club cell protein 16 (CC16) level, However, renal dysfunction (RD) separately raised serum CC16 in our current observation. The purpose of this study was to find the limitation caused by renal dysfunction in the diagnostic performance of CC16 on ARDS in intensive care unit (ICU) patients.

Method: We measured serum CC16 in 479 ICU patients. Patients were divided into six subgroups: control, acute kidney injury (AKI), chronic kidney dysfunction (CKD), ARDS, ARDS+AKI, and ARDS+CKD. The cutoff value, sensitivity and specificity of serum CC16 were assessed by receiver operating characteristic curves.

Result: Serum CC16 increased among the ARDS group when compared to the control group, which helps identify ARDS and predicts the outcome in patients with normal renal function. However, level of serum CC16 was similar among ARDS+AKI, ARDS+CKD, AIK and CKD groups. Consequently, when compare to AKI and CKD, specificity for diagnosing whether ARDS or ARDS with renal failure decreased from 86.62% to 2.82% or 81.70% to 2.12%. Consistently, a cutoff value of 11.57 ng/mL was overturned from previously at 32.77 ng/mL or 33.72 ng/mL. Moreover, its predictive value for mortality is prohibited before 7 day but works after 28 day.

Conclusion: Renal dysfunction limits the specificity, cutoff point, and predictive value at 7-day mortality of CC16 in diagnosing ARDS among ICU patients.

1. Background

Acute respiratory distress syndrome (ARDS) is an acute lung disease with high mortality and morbidity in intensive care units (ICUs). No effective interventions have been established for its treatment due to the limited exploration into the physiological processes. Early correct diagnosis is crucial to determine effective management. However, traditional methods, including PaO_2/FiO_2 and X-ray mentioned in the Berlin definition, actually fall behind the progression of ARDS. Consequently, more than 20 potential biomarkers have been used for the diagnosis and prediction of ARDS in current studies[1], including the club cell protein (CC16).

CC16 is produced by club cells and was first described by the German anatomist Max Clara in 1937 [2]. The bronchial epithelium consists of 80% club cells, such as basal or nonciliated secretory cells, particularly in the distal bronchia[3]. According to previous studies, CC16, as the most abundant secretory protein found in the surface fluids of the airways, was revealed to play an important role in the maintenance and repair of lung airways[4], and it is a potential biomarker of pulmonary injury caused by inhaled ozone, chlorine, and lipopolysaccharides[2].

Five previous studies have evaluated the change of CC16 in ARDS patients. However, the results remain controversial. First, in 2006 a prospective multicenter observational study of 78 critical care patients conducted by the Quebec Critical Care Network found an increase of serum CC16 levels were linked with the onset and negative outcomes of ARDS patients[5]. In addition, Determann et al. discovered increased plasma levels of CC16 in 22 ventilator-associated pneumonia patients who developed ARDS. They found a better diagnostic capacity of CC16 at the cutoff point of 30 ng/ml compared to surfactant Protein D, Krebs von den Lungen, and soluble receptor for advanced glycation end products. It is interesting that increases of CC16 were seen prior to a diagnosis of ARDS [6]. Wutzler et al. further revealed increases of serum CC16 levels accompanied with secondary respiratory complications in patients with multiple injuries[7]. However, Kropski et al. found lower median plasma CC16 levels in ARDS patients than in those with cardiogenic pulmonary edema (22 ng/ml vs. 55 ng/ml) [8]. Furthermore, Ware et al. indicated that lower levels of CC16 (9.2 ng/ml) might help clinicians distinguish ARDS patients from sepsis patients[9]. From the above studies, a contradictory tendency indicated that not only ARDS but other factors influenced serum CC16 levels.

Previously, we found that increasing serum CC16 levels (cutoff point at $\geq$ 33.3 ng/mL) predicted the onset of ARDS and was negatively correlated with the PaO_2/FiO_2 ratio among ARDS patients[10]. However, renal dysfunction (RD) separately raised serum CC16 in our current observation. In the present study, we retrospectively evaluated the limitation caused by RD in the diagnostic performance of serum CC16 on ARDS in ICU patients.

2. Materials And Methods

2.1. Study population

During March 2013 and March 2015, a portion of patients admitted into our ICU were enrolled in our study. The following was the criteria for inclusion for further analysis: 1) patient age over 18 and under 75; 2) ICU stay of more than 12 hours; 3) blood samples were collected less than 6 hours after admission; and 4) diagnosis was clarify before discharge. One of above criterion was not fit when they were not excluded. The Institutional Human Ethics Committee of Baoan Hospital, Nanfang Medical University approved the study protocols employed in this observational study. Written informed consent was obtained from each subject or their legal guardians.

2.2. Data collection and laboratory examination

Values at baseline, including age, gender, blood pressure, body temperature, respiratory rate, heart rate, shock index, and PaO_2/FiO_2 ratio, were collected within 3 hours after admission into the ICU. Seven-day mortality was recorded for all enrolled patients.

Determination of N-terminal of the prohormone brain natriuretic peptide (NT-proBNP), albumin, and serum creatinine were synchronously performed within 3 hours after admission.

All above data were compiled in a Microsoft Office Excel 2003 spreadsheet (Microsoft Corp., Seattle, WA, USA) for subsequent analysis.

2.3. Diagnosis criteria

ARDS diagnosis needed to fit the Berlin definition[11]: 1) acute course, less than 7 days; 2) bilateral opacities consistent with pulmonary edema, as detected by CT or X-ray; and 3) PaO_2/FiO_2 ratio less than 300 mmHg, with ventilation support (Positive End Expiratory Pressure or Continuous Positive Airway Pressure ≥ 5 mmH2O).

Acute kidney injury (AKI) or chronic kidney disease (CKD) needed to fit the clinical practice guidelines of the 2012 Kidney Disease Improving Global Outcomes organization[12]. AKI is defined as an increase in creatinine of ≥ 0.3 mg/dL (26.4 umol/mL) within 48 hours or ≥ 50% above baseline, known or presumed to have occurred within the prior 7 days. CKD is defined as an estimated glomerular filtration rate (Estimated Glomerular Filtration Rate) < 60 mL/min·1.73 m^2 for more than 3 months.

2.4. Subgroup division

Two senior physicians divided the subjects into six subgroups after retrospectively reviewing the diagnosis for each subject based on their clinical conditions within 3 hours after admission: 1) control group: ICU patients without ARDS or RD; 2) AKI group: AKI patients without ARDS; 3) CKD group: CKD patients without ARDS; 4) ARDS group: ARDS patients without RD; 5) ARDS + AKI group: ARDS patients with AKI; and 6) ARDS + CKD group: ARDS patients with CKD.

2.5. Measurement of serum CC16

Blood samples were immediately centrifuged at 3000 rpm for 10 min, and the serum was stored at − 60 °C prior to analysis. The CC16 concentration was determined using an enzyme-linked immunosorbent assay kit (R&D Systems, Minneapolis, MN, USA) following the manufacturer's instructions. A laboratory staff member who had not been provided with the related clinical data performed each assay blindly and in duplicate.

2.6. Statistical analysis

Data were presented as the mean ± standard deviation or median (interquartile range) as indicated. Student's t test or Mann-Whitney U test was used for comparisons between the groups when appropriate based on the normality of the data. Categorical data were compared using the χ^2 or Kruskal-Wallis test. Differences among more than three subgroups were assessed using one-way analysis of variance. Linear correlations among CC16, PaO_2/FiO_2 ratios, albumin, serum creatinine, and NT-proBNP were calculated using the Pearson linear correlation model. Receiver operating characteristic (ROC) curves were introduced to assess the optimal cutoff values, sensitivity, and specificity. A P-value < 0.05 was considered statistically significant. Statistical analyses were performed using the SPSS software package (version 20.0; SPSS Inc., Chicago, IL, USA). Statistical graphs were created using GraphPad Prism 3.0 software (GraphPad Software Inc., La Jolla, CA, USA).

3. Results

3.1 Patient baseline characteristics

A total of 479 critical care patients were recruited into our study, including 230 cases in the control group, 45 cases in the AKI group, 47 cases in the CKD group, 83 cases in the ARDS groups, 61 cases in the ARDS+AKI group, and 13 cases in the ARDS+CKD group. Lower blood pressure along with a higher incidence of pneumonia and sepsis were found in the ARDS group and the ARDS+AKI group compared to the control group. However, higher blood pressure along with a higher proportion of cardiogenic pulmonary edema were discovered in the CKD group and the ARDS+CKD group compared to the control group (Table 1).

3.2 Serum CC16 levels in six subgroups

Serum CC16 was increased among the ARDS group compared to the control group (47.78±19.92 ng/ml vs. 22.23±13.28 ng/ml, *P*=0.001). However, despite having higher levels of serum CC16 than the two above groups, the following groups all had similar levels to each other: the ARDS+AKI group (64.89±20.47 ng/ml), the ARDS+CKD group (72.21±18.63 ng/ml), the AKI group (59.77±26.76 ng/ml), and the CKD group (62.77±25.11ng/ml) (Figure 1).

3.3 Receiver operating characteristic (ROC) curve of serum CC16 levels for the diagnosis of ARDS

We constructed ROC curves to evaluate the diagnostic performance of serum CC16 in critical care patients (Table 2). Although different schemes of ROC curves showed a similar sensitivity, when renal dysfunction presented with baseline characteristics, specificity for diagnosing whether it was ARDS or ARDS with a renal condition (AKI or CKD) decreased from 86.62% to 2.82% (ROC.1 vs. ROC.3) or 81.70% to 2.12% (ROC.2 vs. ROC.4). Consistently, an opposite cutoff value of 11.57 ng/ml was found, a decrease from the previous value of 32.77 ng/ml or 33.72 ng/ml.

3.4 Correlation between serum CC16 levels and other clinical parameters in six subgroups

Compared to the control group, a higher concentration of serum CC16, accompanied with a lower value on the PaO2/FiO2 and albumin, was found in the ARDS group. However, decreased renal function in the AKI, CKD, ARDS+AKI, and ARDS+CKD groups was related to a consistent increase of serum CC16, NT-proBNP, and creatinine compared to the control and ARDS groups. Furthermore, Pearson correlation analysis showed that serum CC16 levels were positively correlated with creatinine (r=0.461, Fig. 3A) and NT-proBNP (r=0.400, Fig.3B) but negatively correlated with PaO_2/FiO_2 (r=0.-277, Fig. 3C) and albumin (r=-0.193, Fig.3D).

3.5 Relationship serum CC16 and outcomes of ICU patients

Higher serum CC16 in all groups presented in the non-survival group with 7-day mortality (68 cases, 14.19%) and 28-day mortality (121 cases, 25.26%) than for those in the survival group (54.99±25.74 ng/mL vs. 38.57±25.76ng/mL; 51.01±25.89* ng/mL vs. 37.49±25.67 ng/mL). However, in the subgroups analysis, renal dysfunction affected the prediction value at 7-day mortality at a similar level of serum CC16 among the AKI group, CKD group, ARDS+AKI group, and ARDS+CKD group (Table 4).

4. Discussion

Consistent with our previous study, increasing serum CC16 helped clinicians identify ARDS in critical care patients with normal renal function. However, renal dysfunction, whether AKI or CKD, synchronously raised serum CC16, resulting in decreased specificity of serum CC16 and overturning the opposite cutoff points. Additionally, its predictive value of the outcome at 7 days disappeared if the patients had renal dysfunction.

The increasing serum CC16 not only depends on the transportation from the bronchoalveolar lavage fluid but also its clearance in the kidney. Several past studies have explored its value in monitoring the permeability of the blood-air barrier, which has a 1000-times concentration gradient constituted from bronchoalveolar lavage fluid (0.5–1.5 mg/L) and serum (10–15 ng/ml) in healthy nonsmokers. Therefore, CC16 has been proved in patents with multiple etiologies, such as chronic exposure to toxicants or severe air pollution[13–15]. However, CC16 has not been responsively synthesized, as evidenced by the negative correlation between serum CC16 and albumin in this study, and synthesis of albumin in response to ARDS can be rapidly reduced until the vascular compartment is repaired[16]. Furthermore, rapid renal clearance of serum CC16 with a half-life of approximately 2–3 hours was previously found to occur via cubilin and/or megalin receptor-mediated endocytosis in the proximal tubule epithelial cells[17]. Andersson et al. found that excretion of CC16 was related to the severity of renal damage, measured by acute dimercaptosuccinic acid scintigraphy. Another clinical study indicated that CC16 can possibly predict creatinine clearance [18]. In addition, in vivo experiments have shown a significant increase in serum CC16 levels of 400 over the basal value after paraquat-induced lung injury[19]; in that study, the elevation of CC16 was mainly determined by the degree of renal impairment. Actually, two above mentioned conditions will increase serum CC16 in critical care patients: destruction of permeability in blood-air barrier and dysfunction of renal clearance[20].

Decreased renal function has been proven to be associated with elevation NT-proBNP in a previous study[21].In our study, worsening renal condition, whether AKI, CKD, ARDS + AKI, or ARDS + CKD, synchronously raised not only CC16 but also NT-proBNP and creatinine. The positive relationship between these renal conditions and CC16, creatinine, and NT-proBNP indicated a higher prevalence of cardiac pulmonary edema in the control group in a study by Kropski et al[8], which also demonstrated baseline characteristics of cardiac pulmonary edema in the CKD or ARDS + CKD group. Our analysis demonstrated that presentation of renal dysfunction weakened the specificity and received an opposite cutoff value of serum CC16 for the diagnosis of ARDS, which might have mostly contributed to the past contradictory gap.

Although CC16 levels were not associated with a one-month clinical respiratory prognosis in a large randomized trial investigation (n = 1200) [22], we found higher serum CC16 still predicted a bad outcome in ICU patients with renal function before 28 days. However, if patients have renal failure, this predictive value is prohibited before 7 days but works after 28 day. In our opinion, a decrease in the serum CC16 might represent a good prognosis, as this could be a sign of repair on the alveolar–capillary barrier in critical care patients, though this needs to be proven through further investigation with adequate data.

Moreover, the biological role of a CC16 increase in the ARDS process is not clear. As an immunoregulatory protein, CC16 executes an anti-inflammatory function by inhibiting phospholipase A2 activation and promoting the expression of inflammatory cascades (IL-1b, IL-8, et al.), TH2 cell differentiation, and the migration of neutrophils and monocytes[23, 24]. Consistent with these studies, Pang demonstrated that recombinant rat CC16 protein inhibits LPS-induced MMP-9 expression and the production of pro-inflammatory cytokines via NF-κB pathway in model of tracheal epithelial cells and RAW264.7 macrophages[25, 26]. She suggested that exogenous supplementation of recombinant CC16 ameliorates cigarette smoke-induced lung inflammation in a murine disease model of chronic obstructive pulmonary disease[27]. Further exploration is needed of CC16's function for critical care patients in the future.

The present study did have a few limitations. First, as a comprehensive ICU, we only enrolled critical care patients but no healthy patients who may have received potential affecting interventions. Although previous studies have revealed a relatively stable median CC16 level in normal controls (5–7 ng/ml), the median CC16 value of critical care patients in our control group appeared to be higher (22.23 ± 13.28 ng/ml), probably because clinical conditions such as mechanical ventilation or primary graft dysfunction might promote the production of CC16[28, 29]. This could have led to an unavoidable selection bias. However, the selection of ICU patients rather than healthy individuals as the control for comparison is clinically meaningful for accurately diagnosing ARDS in the ICU. Second, we only monitored serum CC16 levels on admission and lacked a parallel comparison with other promising biomarkers. Additional valuable clinical investigation related to comparing the CC16 value with other biomarkers for critical care patients needs to be explored in future research with a broader range of patients. Thus, the diagnostic and prognostic values of CC16 in ARDS will require further investigation in a prospective study with a larger sample size.

5. Conclusion

We reported increasing serum CC16 can help identify ARDS and predict the outcome in critical care patients with normal renal function. However, renal dysfunction, whether AKI or CKD, synchronously raises serum CC16, while results in a decrease in its specificity and overturns the optimal cutoff points. Moreover, its predictive value is prohibited before 7 days but works after 28 day.

List Of Abbreviations

AKI, Acute kidney injury; ARDS, acute respiratory distress syndrome; CC16, club cell protein 16; CKD, Chronic kidney dysfunction; ICU, intensive care unit; MAP, mean arterial pressure; NT-proBNP, N-Terminal prohormone brain natriuretic peptide; RD, Renal dysfunction; ROC, Receiver operating characteristic curves; SBP, systolic blood pressure.

Declarations

Ethics approval and consent to participate

The Institutional Human Ethics Committee of Baoan Hospital, Nanfang Medical University approved the study protocols employed in this observational study. Written informed consent was obtained from each subject or their legal guardians.

Consent for publication.

This paper has not been published elsewhere in whole or in part. All authors have read and approved the content, and agree to submit it for consideration for publication in your journal.

Availability of data and materials

The datasets used and/or analyzed during the current study are available

from the corresponding author on reasonable request.

Competing interests

The authors declare no conflict of interest.

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Authors' contributions

Jinle Lin, Wu Jian and Wenwu Zhang participated in the question conception, data analysis, and manuscript draft preparation. Wuyuan Tao, Jianbing Ye, Shiyong Zeng and Xuan Fu abstracted the data collection and Formal Analysis. Qingli Dou, Lijun Wang, and Fang Tian provided critical appraisals of the study and helped with the development of the study question.

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Tables

Table 1. Baseline characteristics of patients at admission

Items	Controls group (n=230)	AKI group (n=45)	CKD group (n=47)	ARDS group (n=83)	ARDS+AKI group (n=61)	ARDS+CKD group (n=13)
Gender, male, n (%)	135 (58.7)	33 (73.3)	24 (51.1)	48 (57.8)	45 (73.8)	8 (61.5)
female, n (%)	95 (41.3)	12 (26.7)	23 (48.9)	35 (42.2)	16 (26.2)	5 (38.5)
Age, years	49.1±18.5	54.1±18.1	64.3±14.5	52.4±20.4	57.0±18.7	57.4±20.4
Body temperature, °C	36.8±0.9	36.7±0.8	36.6±0.7	37.1±0.9	36.1±4.7	36.8±1.2
Heart rate, per min	98.8±24.5	104.4±26.1	89.9±27.3	121.6±103.4*	114.1±26.8*	112.9±28.4
Systolic blood pressure, mmHg	131.6±24.2	127.8±31.6	150.9±36.2	124.5±27.2*	117.9±31.9*	141.3±23.1
Diastolic blood pressure, mmHg	80.59±16.7	76.6±23.0	78.5±21.7	75.8.7±17.14	70.93±22.4	77.9±17.504
Mean arterial pressure, mmHg	97.5±18.1	93.7±24.3	102.6±25.0	92.1±19.2*	86.6±23.7*	100.3±17.59
Shock index	0.78±0.26	0.89±0.45	0.61±0.20#	1.00±0.69*	1.02±0.34*	0.83±0.29#
Pneumonia, n (%)	57 (24.8)	7(15.6)	13 (27.7)	71 (85.5)*	45 (73.8)*	12 (92.3)
Sepsis, n (%)	14 (6.1)	11 (24.4)	5 (10.6)	18 (21.7)*	26(42.6)*	3 (23.1)
CPE	4 (1.7)	4 (8.9)	13 (27.7)#	2 (2.4)	5 (8.2)	7 (53.8)#

Note: Data are analyzed using Student's t test or $\chi 2$ test. AKI, acute kidney injury; CKD, chronic kidney disease; ARDS, acute respiratory distress syndrome; CPE, cardiogenic pulmonary edema; *: Significant difference between the ARDS group or ARDS+AKI group and control group, $P<0.05$; #: Significant difference between CKD group or ARDS+CKD group and control group, $P<0.05$.

Table 2. Receiver operating characteristic (ROC) curves of serum club cell protein 16 for diagnosis of acute respiratory distress syndrome (ARDS) in different schemes

Scheme	AUC	SD	Sensibility	Specificity	Cutoff
ROC.1	0.868	0.023	81.70%	81.74%	32.77
ROC.2	0.909	0.016	86.62%	83.48%	33.72
ROC.3	0.341	0.041	98.78%	2.82%	11.57
ROC.4	0.456	0.038	98.72%	2.12%	11.57
ROC.5	0.780	0.021	87.89%	61.81%	32.77

Note: ROC, receiver operating characteristic; AUC, area under the curve; SD, standard deviation;AKI, acute kidney injury; CKD, chronic kidney disease; ARDS, acute respiratory distress syndrome. *P<0.05

ROC.1 in control group vs. ARDS group;

ROC.2 in control group vs. ARDS group, ARDS+AKD group, and ARDS+CKD group;

ROC.3 in AKI group and CKD group vs. ARDS group;

ROC.4 in AKI group and CKD group vs. ARDS group, ARDS+AKD group, and ARDS+CKD group;

ROC.5 in control group, AKI group and CKD group vs. ARDS group, ARDS+AKD group and ARDS+CKD group.

Table 3. Serum CC16 and other clinical parameters in five subgroups

Items	Control group (n=230)	AKI group (n=45)	CKD group (n=47)	ARDS group (n=83)	ARDS+AKI group (n=61)	ARDS+CKD group (n=13)
CC16 (ng/mL)	22.23±13.28	59.77±26.76	62.27±25.11	47.78±19.92*	64.89±20.47	72.21±18.63
PaO_2/FiO_2	353.50±143.52	345.09±147.46	291.61±136.76	204.19±84.71*	178.19±84.92	205.77±137.15
Creatinine (mg/mL)	67.48±22.68	358.23±335.55#	583.15±438.233#	72.03±24.19	225.90±153.48	633.05±630.61#
NT-proBNP (pg/mL)	267.68±539.76	983.97±1020.23#	1687.03±1389.56#	421.74±756.90	1022.99±1313.93	2402.14±1662.05#
Albumin (g/mL)	33.48±8.10	29.83±7.23	31.92±6.17	28.21±6.44*	29.22±9.05	32.26±6.32

Note: CC16, club cell protein 16; NT-proBNP, N-terminal of the prohormone brain natriuretic peptide; AKI, acute kidney injury; CKD, chronic kidney disease; ARDS, acute respiratory distress syndrome;*: Significant difference value between the ARDS group and control group; #: Significant difference value in the AKI group, CKD group, ARDS+AKI group, and ARDS+CKD group compared to the control group or ARDS group.

Table 4. Relationship between serum CC16 and outcome of intensive care unit (ICU) patients

Items		Control group vs. ARDS group	AKI and CKD group vs. ARDS+AKI and ARDS+CKD group	All groups
7-day mortality, *n* (%)		18 (7.8) vs.17 (20.5)	9 (20.0) and 5 (10.6) vs. 17 (27.9) and 2 (15.4)	68 (14.19)
CC16(ng/mL)	Non-survival group	40.27±22.61*	70.41±19.20	54.99±25.74*
	Survival group	27.57±18.05	61.58±24.28	38.57±25.76
28-day mortality, n (%)		32 (13.9) vs.33 (39.8)	15 (33.3) and 11 (23.4) vs. 27 (44.3) and 3 (23.1)	121□25.26□
CC16(ng/mL)	Non-survival group	37.16±20.62*	66.99±22.04*	51.01±25.89*
	Survival group	26.88±18.01	61.42±24.27	37.49±25.67

Note: CC16, club cell protein 16; AKI, acute kidney injury; CKD, chronic kidney disease; ARDS, acute respiratory distress syndrome;* significant difference value between Non-survival group and Survival group; P <0.05.

Figures

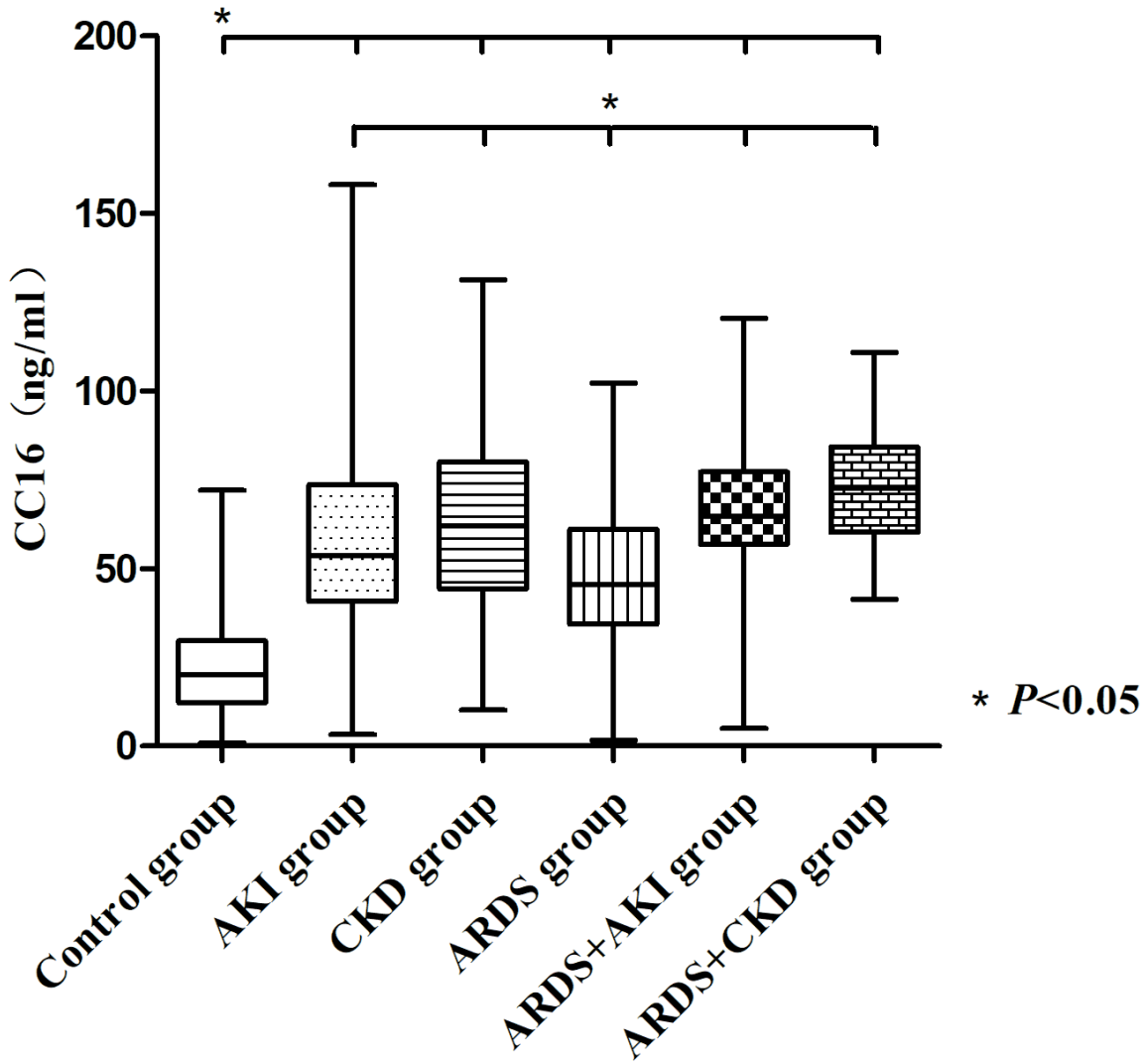

Figure 1

Comparison of the serum CC16 levels between the control group, AKI group, CKD group, ARDS group, ARDS+AKI group, and ARDS+CKD group. *:P-value<0.05.

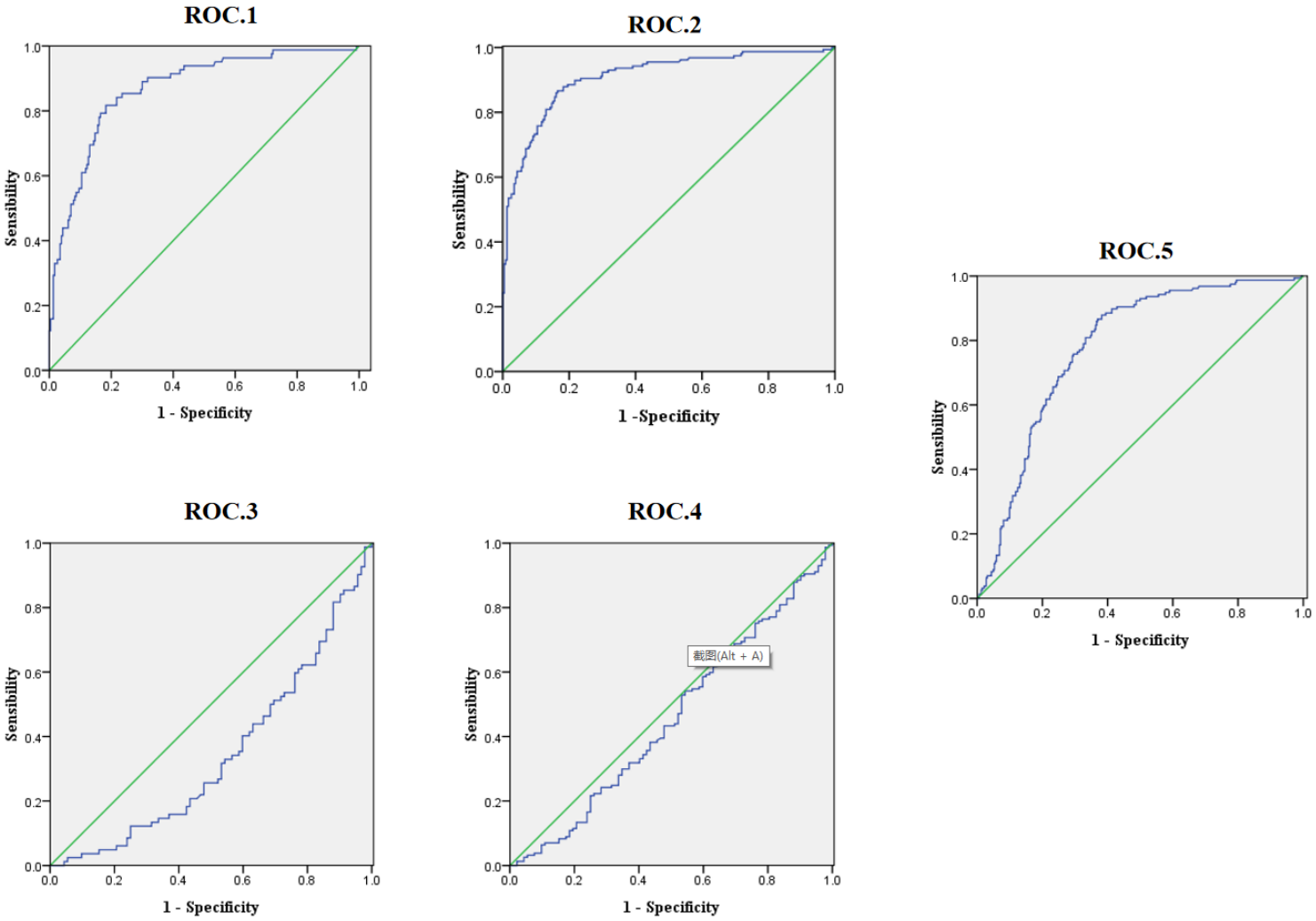

Figure 2

Five ROC curves of serum CC16 for diagnosing ARDS in critical care patients: ROC.1 in the control group vs. the ARDS group; ROC.2 in the control group vs. the ARDS group, ARDS+AKD group, and ARDS+CKD group; ROC.3 in the AKI group and CKD group vs. the ARDS group; ROC.4 in the AKI group and CKD group vs. the ARDS group, ARDS+AKD group, and ARDS+CKD group; ROC.5 in all groups.

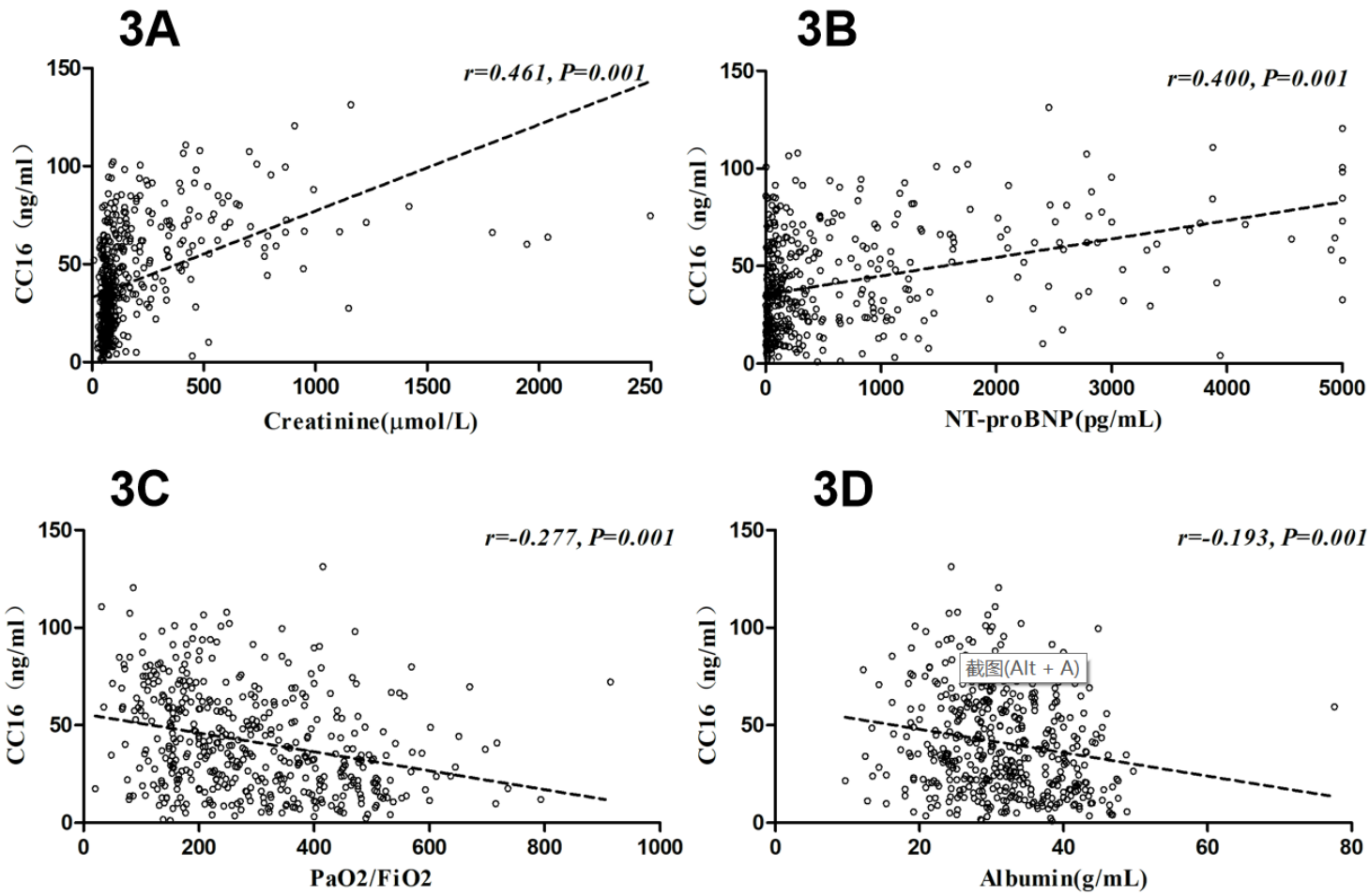

Figure 3

3A. A positive correlation between the serum CC16 levels and serum creatinine in all groups. 3B. A positive correlation between the serum CC16 levels and NT-proBNP in all groups; 3C. A negative correlation between the serum CC16 levels and PaO2/FiO2 in all groups. 3D. A negative correlation between the serum CC16 levels and albumin in all groups.