Identification of RAGE and OSM as new prognosis biomarkers of severe pneumonia

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Abstract

Background

Patients with severe pneumonia complicated with hypoxic respiratory failure often associated with increased morbidity and mortality rates. It is critical to discover more sensitive and specific markers for early identification of such high risk patients thus specific and timely treatment can be adjusted.

Methods

This retrospective study was performed in the respiratory intensive care unit (RICU) of Nanjing First Hospital and Jinling Hospital, Nanjing Medical University. Clinical data of patients admitted to the RICU and diagnosed with pneumonia from January 2017 to October 2019 was retrospectively reviewed. The eligible patients were classified into hypoxemia and non hypoxemia groups according to oxygenation index of 250 mmHg. In the meantime, the same cohort was separated into survival and deceased groups after 30 days post hospital admission. The related risk factors in these two classifications were examined separately.

Results

A total of 828 patients were screened for eligibility, and eventually 130 patients with pneumonia were included in our final analysis. Among the patients, 16 passed away despite exhausting standard treatments. The comparison between hypoxemia and non hypoxemia groups suggested that gender, diabetes mellitus status, count of white blood cell(WBC), neutrophils, neutrophils/Lymphocyte, lactic acid, creatinine, D-dimer, procalcitonin (PCT), C-reactive protein (CRP), PH, Lymphocyte, albumin and RAGE were significantly different.

Conclusions

Previous studies have suggested that the APACHE II score, LIS, SOFA, Nutric scores, WBC, neutrophils, lymphocyte counts and albumin levels were independent risk factors for severe pneumonia. Our study indicated that RAGE should be a new biomarker to predict poor prognosis in pneumonia. In addition, we also showed that LIS, SOFA, lactate, lymphocyte, platelet, BUN, total bilirubin, and PCT levels before treatment were independent factors that associated with 30 days survival rate. In addition, we proposed that OSM should be considered as a new prognosis marker for pneumonia patients.
Background
Pneumonia is a common disease that usually associates with benign outcome. However, patients with severe disease often accompanied with hypoxic respiratory failure and associate with high morbidity and mortality rates. Multiple biomarkers for such patients have been identified in past decades and have been proved repeatedly for its importance in guiding proper clinic treatments.

There are many clinical scores and laboratory indicators that have been used in real world clinical practice for pneumonia diagnosis and treatment. For instance, scores such as acute physiology, age, chronic health evaluation (APACHE II), lung injury score (LIS), sequential organ failure assessment (SOFA), and The Nutrition Risk in Critically ill score (NUTRIC score) are often applied in intensive care unit. Some lab results, for instance, white blood cell count (WBC), hepatic, renal, cardiac function test, coagulation analysis, C-reactive protein (CRP), procalcitonin(PCT) level, and T cells subtypes have all been proved very important in understanding the disease process and evaluating treatment. Even though the pathogenesis of pneumonia has been well examined, studies about discovering specific and sensitive markers for early identification of patients with increased risks developing severe disease, especially for those who complicated with hypoxic respiratory failure, are relatively insufficient. In addition, it has been known that comorbidities like cerebrovascular disease, diabetes mellitus, coronary heart disease, chronic renal damage, and nasopharyngeal carcinoma are risk factors of pneumonia progression. However, there predictive value in disease prognosis have not been examined before.

Severe pneumonia causes diffuse lung injury involving different types of cells. Type I epithelial cells cover 90–95% of the alveolar surface and contribute to the barrier integrity. Receptor for advanced glycation end products (RAGE) is a 35 kDa protein from the immunoglobulin superfamily and propagates the inflammatory response via NF-KB. RAGE is a marker of type I alveolar epithelial cell injury in rats and in patients with acute respiratory distress syndrome (ARDS). In addition, the plasma level of RAGE was decreased in lung-protective ventilation in patients undergoing major abdominal surgery compared to non-protective ventilation. There was a study suggested higher
baseline plasma level of RAGE was associated with increased mortality\textsuperscript{12}. However, the relationship between the change of RAGE during disease course and prognosis is still understudied. The damage of endothelial cells increased permeability to liquid and protein and results in edema in interstitium space\textsuperscript{13}. Endothelial cells express Oncostatin M (OSM) receptors, which is a member of the IL-6 family. Neutrophil-released OSM affects endothelial cellular function under both physiological and pathological conditions\textsuperscript{14}. However, despite its importance in disease pathogenesis, whether OSM can be used as a biomarker that predicts the disease severity and prognosis has not been studied.

In this study, we examined the possibility of RAGE and OSM as new biomarkers predicting disease outcomes in pneumonia. Furthermore, we combined RAGE and OSM with other clinical and laboratory scores in order to improve the accuracy of the prediction.

Methods
This retrospective, observational study was performed in the respiratory intensive care unit (RICU) of Nanjing First Hospital and Jinling Hospital, Nanjing Medical University. This study was approved by the Jinling Hospital and Nanjing First Hospital Ethics Committee (Approval Number: JLYY: 2013021). All clinical data was retrospectively reviewed for patients admitted to the RICU for pneumonia from January 2017 to October 2019.

Study Design
Patients were separated into survival and non-survival groups. Patients with pneumonia that had different outcomes were compared and analyzed in terms of clinical, laboratory and prognostic characteristics on the first day and the seventh day after hospital admission. We then analyzed the related risk factors of disease mortality.

Participants
All patients included in this study were well informed and signed consents for all the tests and treatments were collected at the beginning of this study. Patients included in our study were presenting at least one acute symptom (breathlessness, cough or fever), and had recent lung infiltrates based on chest high resolution computed tomography.
Patients with other lung complications such as tuberculosis, acute pulmonary embolism, congenital heart disease, and un-treated aggressive carcinoma were excluded from this study.

Clinical Manifestations And Laboratory Measurements
All patients’ clinical data including disease course, medical history, smoking history, drinking history, physical signs and vital signs, hospitalization time, prognosis, as well as medication histories were collected on the first day and the seventh day after the RICU admission. We also collected individual treatment history for each patient, such as detail of oxygen usage and MV setting. At the same time, we also recorded blood cell counting, hepatic, renal and cardiac function test, coagulation analysis, CRP, PCT, OSM, RAGE, and T cells subtypes results.

Clinical And Laboratory Measurements And Recordings
LIS, NUTRIC score, gas exchange, organ failure and other indicators were used to assess disease severity upon patients’ admission to RICU. Specifically, The LIS score was calculated from four aspects: oxygenation index, the area of pulmonary infiltration on chest X ray, positive end expiratory pressure(PEEP) and Lung compliance. The probability of severe lung injury increase as the scores go up. The NUTRIC score was calculated based on age, APACHE II score, SOFA, concomitant diseases, time from admission to ICU, and IL-6. APACHE II is a disease classification system used to identify acutely ill patients with poor prognosis via 14 basic physiologic principles. There are: (I) predisposing conditions, including shock, aspiration, sepsis, pneumonia, high-risk surgery, and high-risk trauma; (II) risk modifiers, containing alcohol abuse, body mass index (BMI) > 30 kg/m2, hypoalbuminemia, chemotherapy, FiO2 > 35%, respiratory rate > 30 bpm, SpO2 < 95%, pH < 7.35, and diabetes mellitus\textsuperscript{15}. SOFA is an organ failure assessment system, which is graded from 0 to 4 according to the degree of dysfunction in each of 6 organ systems (central nervous system, cardiovascular, respiratory, renal, liver and coagulation)\textsuperscript{16}. However, it is important to point out that due the limitation of this study, the measurement of IL-6 was amid in this study.

Statistical analysis
Statistical analysis was performed using SPSS. Continuous variables were presented as the mean with standard deviation and compared by independent-sample Student’s t-test. Categorical variables were summarized as frequency and percentages, and the differences between groups were analyzed using
Chi-square test and Man-Whitney test. We considered \( p < 0.05 \) as statistically significant for all analyses.

Risk factors were analyzed by single factor Logistic regression assay. The area under receiver operating characteristic (ROC) curves indicated a strong predictive power for the biomarkers' area, which represented the largest area under the curve (AUC). The cut-off points of biomarkers obtained for the calculated area had the maximum sum of sensitivity and specificity for predicting mortality and severity of pneumonia. The relationships between survival and severity of pneumonia with different biomarkers were represented by the Pearson product-moment coefficient of correlation method.

Results
A total of 828 patients were screened for eligibility, and eventually 130 patients were enrolled in our final analysis. Enrolled patients were then divided into two groups according to the oxygenation index of 250 mmHg. Among the patients, 16 were dead, and 112 survived.

1. Comparing the effect of gender and comorbidities on oxygenation index between the two groups of oxygenation index \( \leq 250 \) mmHg and oxygenation index > 250 mmHg.

Major comorbidities identified in both groups were presented as above. They were no difference of percentages of having cerebrovascular disease, coronary heart disease, chronic renal damage, and nasopharyngeal carcinoma between two groups. However, our data suggested that the percentage of having diabetes mellitus in the hypoxia group was higher and the difference of sex ratio indicated that men more likely developed hypoxemia (Table 1).

| Group | Gender | Z | P  |
|-------|--------|---|----|
|       | oxygenation index > 250 mmHg | oxygenation index ≤ 250 mmHg |     |
| Gender | W21(54),M33(54) | W16(76),M60(76) | -2.212 | 0.027 |
| Cerebrovascular disease | 11 | 26 | -1.717 | 0.086 |
| Diabetes mellitus | 7 | 21 | -1.997 | 0.046 |
| Coronary heart disease | 19 | 31 | -0.645 | 0.519 |
| Chronic renal damage | 4 | 12 | -1.428 | 0.153 |
| Treated nasopharyngeal carcinoma | 0 | 2 | -1.197 | 0.231 |

2. Comparing the effect of age and clinical scores on oxygenation index between the two groups of
oxygenation index ≤ 250 mmHg and oxygenation index > 250 mmHg.

Age and different clinical scores were calculated and compared between two groups (Table 2). There was no difference of age between two groups. However, the APACHE II, LIS, SOFA and NUTRIC scores were higher in the group of oxygenation index ≤ 250 mmHg compared to the group of oxygenation index > 250 mmHg.

| Table 2 | Age, APACHE II, LIPS, SOFA and Nutric scores (´x̄±s) |
|---------|------------------------------------------------------|
| Group   | oxygenation index > 250 mmHg (n = 54) | oxygenation index ≤ 250 mmHg (n = 76) | t     | P     |
| Age     | 69.63±16.17                                      | 72.78±14.94                             | -1.144 | 0.255 |
| APACHE II | 16.97±6.12                                           | 20.03±6.84                              | -2.17  | 0.032 |
| LIS     | 3.13±2.24(32)                                              | 7.04±2.78(74)                           | -7.032 | 0.0001|
| SOFA    | 2.67±2.60(30)                                              | 6.34±3.41(74)                           | -5.294 | 0.0001|
| NUTRIC score | 2.60±2.71(53)                                             | 5.46±1.89(76)                           | -7.069 | 0.0001|

3 Comparing the effect of clinical markers on oxygenation index between the two groups of oxygenation index ≤ 250 mmHg and oxygenation index > 250 mmHg.

As indicated in Table 3, compared with patients with oxygenation index > 250 mmHg, the count of White blood cell (WBC), neutrophils, neutrophils/Lymphocyte ratio, lactic acid, creatinine, D-dimer, PCT, CRP and RAGE levels were significantly higher in patients with oxygenation index ≤ 250 mmHg, while the PH, Lymphocyte and albumin levels were significantly lower (P < 0.05). However, there were significant differences in PaCO2, hematocrit, hemoglobin, platelet, erythrocyte, Urea nitrogen(BUN), Lactate dehydrogenase(LDH), total bilirubin, Prothrombin time(PT), activated partial thromboplastin time (APTT), Fibrinogen (Fib) and OSM level between two groups.
Table 3
Laboratory examination results at RICU admission (x ¯ ± SD)

| at admission | Group | t | p |
|--------------|-------|---|---|
| Immunophenotyping | oxygenation index > 250 mmHg (n = 54) | oxygenation index ≤ 250 mmHg (n = 76) | 1.324 | 0.189 |
| CD4 | 462.79 (28) | 421.08 (65) | 0.189 | 0.990 |
| CD8 | 259.79 (28) | 259.49 (65) | 0.012 | 0.990 |
| CD3 | 809.93 (28) | 764.83 (64) | 0.931 | 0.354 |
| Arterial blood gas | pH | 7.44 (0.45) | 7.41 (0.09) | 1.850 | 0.067 |
| PaCO2 (mmHg) | 38.56 (7.05) | 41.66 (17.25) | -1.206 | 0.230 |
| Lac | 1.47 (0.80) | 1.88 (1.39) | -1.880 | 0.063 |
| Blood cell analysis | WBC (10⁹/L) | 8.09 (4.16) | 10.71 (6.10) | -2.717 | 0.008 |
| Neutrophils (10⁹/L) | 6.09 (3.83) | 9.31 (5.71) | -3.581 | 0.000 |
| Lymphocyte (10⁹/L) | 1.28 (0.80) | 0.86 (0.53) | 3.555 | 0.001 |
| Hematocrit (%) | 0.37 (0.07) | 0.37 (0.08) | -0.277 | 0.782 |
| Hemoglobin (g/L) | 122.60 (21.85) | 121.04 (27.05) | 0.349 | 0.728 |
| Platelet (10⁹/L) | 199.74 (74.08) | 199.16 (105.09) | 0.034 | 0.973 |
| Biochemical analysis | Albumin (g/L) | 35.80 (7.70) | 30.38 (4.53) | 4.962 | 0.000 |
| BUN (mmol/L) | 6.79 (5.08) | 19.66 (59.82) | -1.545 | 0.120 |
| Creatinine (µmol/L) | 87.43 (96.15) | 123.60 (102.80) | -2.007 | 0.047 |
| LDH (IU/L) | 122.60 (218.85) | 121.04 (270.57) | 0.349 | 0.728 |
| Total bilirubin (µmol/L) | 12.17 (5.63) | 12.45 (10.06) | -0.184 | 0.854 |
| Coagulation analysis | PT (s) | 12.61 (4.10) | 12.85 (2.48) | -0.405 | 0.686 |
| APTT (s) | 29.97 (5.91) | 30.67 (7.01) | -0.587 | 0.558 |
| FIB (g/L) | 4.54 (1.60) | 5.35 (4.69) | -1.194 | 0.235 |
| D-dimer (µg/ml) | 1.31 (1.91) | 5.66 (8.68) | -3.549 | 0.001 |
| Inflammatory biomarkers | Procalcitonin (ng/mL) | 0.61 (1.60) | 6.98 (20.91) | -2.105 | 0.037 |
| CRP (ng/mL) | 59.83 (64.33) | 113.59 (96.67) | -3.487 | 0.001 |
| OSM (pg/mg) | 54.85 (58.64) | 80.32 (110.43) | -1.530 | 0.128 |
| RAGE (pg/ml) | 632.62 (469.73) | 1034.26 (1068.24) | -2.563 | 0.012 |

4. Comparing the effect of gender and comorbidities on survival between the two groups of survival and non survival.

We discovered no difference in gender, cerebrovascular disease, diabetes mellitus, coronary heart disease, chronic renal damage, and nasopharyngeal carcinoma between two groups. (Table 4)

Table 4
Gender and comorbidities between survival and non survival patients

| Group | Z | p |
|-------|---|---|
| Gender | non survival n = 16 | survival n = 114 | -0.916 | 0.360 |
| Cerebrovascular disease | W3(16), M13(16) | W34(114), M80(114) | -2.095 | 0.086 |
| Diabetes mellitus | 5 | 23 | -1.005 | 0.315 |
| Coronary heart disease | 9 | 41 | -1.356 | 0.120 |
| Chronic renal damage | 3 | 13 | -0.834 | 0.404 |
| Treated nasopharyngeal carcinoma | 0 | 2 | -0.532 | 0.595 |
5. Comparing the effect of age and clinical scores on survival between the two groups of survival and non survival.

Our data suggested that there was no difference in Age and APACHE II score between two groups. However, LIS, SOFA, NUTRIC scores were significantly higher in the non-survival group compared to the survival group (P < 0.05). (Table 5)

| Group            | non survival (n = 16) | survival (n = 114) | t  | p   |
|------------------|-----------------------|--------------------|----|-----|
| Age              | 73.44(12.36)          | 71.19(15.89)       | 0.542 | 0.589 |
| APACHE II        | 20.93(6.02)           | 18.77(6.84)        | 1.148 | 0.254 |
| LIS              | 7.56(3.93)            | 5.56(2.95)         | 2.376 | 0.019 |
| SOFA             | 7.69(5.04)            | 4.84(3.11)         | 3.022 | 0.003 |
| NUTRIC score     | 7.69(5.04)            | 4.09(2.68)         | 2.292 | 0.024 |

6. Comparing the effect of clinical markers on survival between the two groups of survival and non survival.

As indicated in Table 6, compared to survival patients, the levels of lactic acid, BUN, total bilirubin, PCT and OSM were significantly higher in non-survival patients, while lab results showed no differences between these two groups.
### Table 6
Laboratory examination results between survival and non survival patients (\(x\̇s\))

| Before treatment | Group | \(t\) | \(P\) |
|------------------|-------|-------|-------|
|                  | non survival (\(n = 16\)) | survival (\(n = 114\)) |       |
| Immunophenotyping | CD4   | 381.33 | 35.13(12) | 441.38 | 147.94(81) | -1.394 | 0.167 |
|                  | CD8   | 221.50 | 26.78(12) | 265.22 | 110.56(81) | -1.358 | 0.178 |
|                  | CD3   | 681.33 | 77.79(12) | 793.14 | 223.77(80) | -1.708 | 0.091 |
| Arterial blood gas | pH    | 7.44(16) | 0.08(12) | 7.42(110) | 0.08(110) | 1.074 | 0.285 |
|                  | PaCO2 (mmHg) | 39.59(16) | 8.41(12) | 40.55(110) | 14.83(110) | -0.252 | 0.801 |
|                  | Lac   | 2.46(16) | 2.13(12) | 1.61(110) | 0.98(110) | 2.676 | 0.008 |
| Blood cell analysis | WBC (10^9/L) | 8.30(16) | 4.63(12) | 9.83(110) | 5.63(110) | -1.034 | 0.303 |
|                  | Neutrophils (10^9/L) | 7.26(16) | 4.55(12) | 8.09(110) | 5.36(110) | -0.587 | 0.558 |
|                  | Lymphocyte (10^9/L) | 0.73(16) | 0.59(12) | 1.078(110) | 0.69(110) | -1.941 | 0.054 |
|                  | Hematocrit (%) | 0.35(16) | 0.08(12) | 0.37(110) | 0.08(110) | -0.852 | 0.396 |
|                  | Hemoglobin (g/L) | 115.56(16) | 25.18(12) | 122.55(110) | 24.93(110) | -1.048 | 0.297 |
|                  | Platelet (10^12/L) | 139.88(16) | 71.63(12) | 207.82(110) | 92.05(110) | -2.799 | 0.006 |
|                  | Erythrocyte (10^12/L) | 3.74(16) | 0.72(12) | 4.13(110) | 0.89(110) | -1.691 | 0.093 |
|                  | Neutrophils/Lymphocyte (%) | 13.94(16) | 12.15(12) | 12.63(110) | 16.89(110) | 0.298 | 0.766 |
| Biochemical analysis | Albumin (g/L) | 33.59(16) | 11.41(12) | 32.43(110) | 5.59(110) | 0.664 | 0.508 |
|                  | BUN (mmol/L) | 48.27(16) | 128.46(12) | 9.60(110) | 7.56(110) | 3.223 | 0.002 |
|                  | Creatinine (µmol/L) | 113.06(16) | 81.79(12) | 108.31(110) | 104.13(110) | 0.175 | 0.862 |
|                  | LDH (IU/L) | 710.81(16) | 948.51(12) | 441.72(110) | 490.33(110) | 1.780 | 0.071 |
|                  | Total bilirubin (µmol/L) | 17.49(16) | 12.15(12) | 11.59(110) | 7.65(110) | 2.651 | 0.009 |
| Coagulation analysis | PT (s) | 13.23(16) | 3.25(12) | 12.68(110) | 3.23(110) | 0.626 | 0.533 |
|                  | APTT(s) | 31.43(16) | 9.16(12) | 30.24(110) | 6.15(110) | 0.675 | 0.501 |
|                  | FIB (g/L) | 3.85(16) | 2.23(12) | 5.19(110) | 3.91(110) | -1.328 | 0.187 |
|                  | D-dimer | 6.12(16) | 8.74(12) | 5.87(110) | 6.83(110) | 1.342 | 0.182 |
|                  | Inflammatory biomarkers | Procalcitonin (ng/mL) | 14.68(15) | 36.15(12) | 3.04(110) | 11.28(105) | 2.545 | 0.012 |
|                  |                  | CRP (ng/L) | 88.27(16) | 107.42(12) | 92.54(110) | 86.45(110) | -0.180 | 0.857 |
|                  |                  | OSM (pg/µg) | 142.63(15) | 196.72(12) | 59.92(110) | 64.15(110) | 3.366 | 0.001 |
|                  |                  | RAGE (pg/ml) | 1099.89(15) | 810.56(12) | 835.41(110) | 898.62(110) | 1.082 | 0.281 |

7. Comparing the effect of clinical markers after treatment on survival between the two groups of survival and non survival.

As presented in Table 7 and Table 8, the PH, lymphocyte, albumin and platelet levels increased and the number of neutrophils, RBC, hemoglobin, hematocrit, creatinine, total bilirubin, CRP, PCT, OSM, RAGE and neutrophils/lymphocyte ratio decreased in the group of survival patients after treatment. It is important to point out that even though CRP and PaCO2 raised similarly to the survival group, the PH went lower in the group of non-survival after treatment (\(P < 0.05\)). Meanwhile, Lac, WBC, neutrophils, lymphocyte, RBC, hemoglobin, hematocrit, platelet, albumin, creatinine, BUN, total bilirubin, PT, APTT, FiB, PCT, OSM, RAGE and neutrophils/lymphocyte ratio did not change significantly after treatment in the non-survival group.
Comparing the correlation between the above markers with oxygenation index and survival.

Next, we determined the relationship between scores of APACHEII, LIS, SOFA, NUTRIC, PH, lac, WBC, neutrophils, lymphocyte, neutrophils/lymphocyte, creatinine, RAGE, and albumin with oxygenation index individually, using the single factor Logistic regression and 95% confidence interval. Our data suggested that the oxygenation index was related to APACHEII, LIS, SOFA, NUTRIC scores, WBC, neutrophils, lymphocyte, RAGE, and albumin levels (P < 0.05). In addition, with the same method, we discovered that LIS, SOFA, NUTRIC scores, lac, lymphocyte, platelet, BUN, total bilirubin, PCT and OSM...
levels were related to mortality rate ($P < 0.05$).

9 Examine the correlation between oxygenation index and RAGE.

In order to examine the relationship between oxygenation index and the RAGE, we calculated the Pearson Correlation Coefficient with $−0.228$, $P = 0.001$ (Fig. 1). Indicating a meaningful negative correlation between RAGE and oxygenation index.

10. Examining the correlation between pneumonia mortality with OSM.

OSM level before treatment was associated with the pneumonia mortality. The Pearson correlation coefficient was $−0.228$, and $P = 0.001$ (Fig. 2).

We examined all markers, and found BUN, bilirubin and platelet before treatment, CRP and PCT after treatment were correlate with survival. Furthermore we calculated the cutoff value of them.

11. Examining the cutoff value of BUN before treatment.

The AUC of BUN before treatment was calculated to be $0.738$ (95% CI: $0.607$–$0.869$, $P = 0.002$) with a cutoff point of $9.22$ mmol/L yielding a sensitivity and specificity of $81.3\%$ and $64.3\%$, respectively (Fig. 3).

12. Examining the cutoff value of bilirubin and platelet before treatment.

ROC curves of serum total bilirubin and platelet resulted in an AUC of $0.69$ (95% CI: $0.557$–$0.824$, $P = 0.014$) and $0.287$ (95% CI: $0.127$–$0.447$, $P = 0.006$), respectively, using a cutoff value of $8.55$ umol/L for serum total bilirubin with a sensitivity of $93.8\%$ and specificity of $40.9\%$, and a cutoff value of $28\times10^{9}/L$ for platelet with a sensitivity and specificity of $100\%$ and $0\%$ (Fig. 4).

13. Examining the cutoff value of CRP and PCT after treatment.

In order to identify the risk factors for death, we calculated the AUC of CRP and PCT after treatment which were $0.868$ (95% CI: $0.712$–$1$, $P = 0.003$) and $0.855$ (95% CI: $0.733$–$0.977$, $P = 0.004$) with cutoff values being $70.15$ mg/L and $0.24$ ng/ml respectively. The sensitivity/specificity was $83.3\%/87.9\%$ and $100\%/72.4\%$ respectively. (Fig. 5).

Discussion

In this retrospective study, we discovered that pneumonia with lower oxygenation index was associated with gender, diabetes mellitus, APACHE II score, LIS, SOFA, Nutric score, PH, lactic acid,
WBC, neutrophils, lymphocyte, neutrophils/lymphocyte count, creatinine, D-Dimer, PCT, CRP, RAGE and albumin levels. Among these factors, the APACHE II score, LIS, SOFA, Nutric scores, WBC, neutrophils, lymphocyte count, RAGE and albumin levels were independent risk factors for severe pneumonia. Several reasons can contribute to the lower oxygenation index. For instance, it could be due to unbalanced ventilation and blood flow ratio, pulmonary shunts, and decreased pulmonary diffusion function. In our study, we excluded patients with acute pulmonary embolism who were much likely suffering from pulmonary shunts.

In this study, we discovered that patients with diabetes mellitus were more likely developed into severe conditions. This finding was inconsistent with some other studies, which suggested that hypoxemia was not associated with diabetes mellitus\textsuperscript{17}. We believe the impaired microvasculature in diabetes mellitus patients deteriorated the already impaired oxygenation in lung and resulted in a lower oxygenation index\textsuperscript{18,19}. However, more studies including larger population are needed to further examine this association.

In this study, we also examined the association between WBC, neutrophils and lymphocyte counts and the disease prognosis for their distinct role in the pathogenesis\textsuperscript{20,21}. We discovered that the count of White blood cell (WBC), neutrophils, neutrophils/Lymphocyte ratio were significantly higher in patients with oxygenation index $\leq 250$ mmHg, while the PH, Lymphocyte and albumin levels were significantly lower (P $< 0.05$). This is consistent with previous studies and our clinical observations\textsuperscript{22}.

Among the markers, we found that the increased serum RAGE level was an independent factor that related to hypoxemia in pneumonia patients (the Pearson Correlation Coefficient $-0.228$, P = 0.001). RAGE is constitutively highly expressed in type 1 and type 2 alveolar epithelial cells and vascular smooth muscle cells in lung\textsuperscript{23}. It has been well accepted that RAGE is defined as a specific marker of ARDS\textsuperscript{11}. Our data suggested that RAGE might be a new biomarker to predict hypoxemia of pneumonia even before the on-set of ARDS.

Furthermore, we classified the patients into survival group and non-survival group. Severe pneumonia is one of the major causes for ICU admission and associates with high morbidity and mortality rates.
Early identification of patients of higher risk could potentially help for early intervention and prevent severe pneumonia patients from death. We found that LIS, SOFA, Nutric scores, lactate, lymphocyte, platelet, erythrocyte, BUN, LDH, total bilirubin, PCT, and OSM levels were significance difference between two groups. While the LIS, SOFA scores, lactate, lymphocyte, platelet, BUN, total bilirubin, PCT, and OSM levels before treatment were proved to be independent predict factors that associated with death. Consistently, some studies had also recognized that lactate and lymphocyte could predict disease.4,5

The lung is a site of platelet biogenesis and a reservoir for hematopoietic progenitors.6 Our result showed that lower platelet count was related to death. Previous study suggested that BUN was independently associated with mortality in critically ill patients that was in consistent with our findings.7 The cutoff value of BUN was set to 9.22 mmol/L, and the sensitivity and specificity to predicting death was 81.3% and 64.3% in our study. Previous study also suggested that increased serum bilirubin level was associated with mortality.8 The cutoff value of serum bilirubin was set to 8.55umol/L in our study and the sensitivity and specificity of possibility of death was 93.8% and 40.9% respectively. We found out that PCT and CRP levels after treatment were independent factors associated with 30 day-death, but not the levels before treatment. The single factor logistic regression analysis showed that PCT and CRP after treatment were risk factors for death, which had a sensitivity of 100% and specificity of 72.4% with a cutoff value of 0.24 ng/ml, and a sensitivity of 83.3% and specificity of 87.9% with a cutoff value of 70.15 mg/L respectively. Some studies found PCT at admission was not an independent predictor of 30-day mortality in both of elderly and younger patients.24 While other studies thought elevated value of PCT at admission had moderate accuracy to identify poor outcome in septic patients.25 However, we were among the very few groups that studied the relationship of PCT after treatment with disease morbidity.

Another biomarker was OSM, which was one of the IL-6 family cytokines. The secretion of OSM increases in response to bacterial stimuli. However, its’ role in pneumonia was currently understudied. In this study, we demonstrated that the increased level of serum OSM at admission was
associated with 30 day-death. We believed that OSM level in pneumonia is an important signal for increased chemokine induction that further mediated neutrophil recruitment which finally resulted in more serious inflammation\textsuperscript{26}. OSM is also associated with increased vessel permeability for its’ capability of destroying endothelial barrier. In addition, it has been known that OSM can also stimulate fibroblast activation and contribute to multiple organ dysfunction during severe infections\textsuperscript{27}. Our study suggested that the level of OSM was associated with disease prognosis. Future studies are needed to further confirm the predictive value of OSM in real world.

Furthermore we analyzed the changes of those biomarkers between survival group and non-survival group after one week of standard treatment. We discovered that PH, neutrophils, lymphocyte, erythrocyte, hemoglobin, hematocrit, platelet counts, neutrophils/lymphocyte ratio, creatinine, total bilirubin, PCT, CRP, RAGE, OSM and albumin levels were improved in the survival group. However, lactic acid, WBC, neutrophils, lymphocyte, erythrocyte, hemoglobin, hematocrit, platelet count, neutrophils/lymphocyte ratio, creatinine, total bilirubin, PT, APTT, Fib, PCT, RAGE, OSM and albumin levels were not improved in non-survival patients.

In summary, our data suggested that the importance of LIS, SOFA, Nutric scores, lactate, lymphocyte, platelet, erythrocyte, BUN, LDH, total bilirubin, PCT, and OSM levels at admission should be appreciated when predicting disease prognosis. After one week treatment, the lactic acid, WBC, neutrophils, lymphocyte, erythrocyte, hemoglobin, hematocrit, neutrophils/lymphocyte ratio, platelet, creatinine, total bilirubin, PT, APTT, Fib, PCT, RAGE, OSM and albumin levels should be examined to further identify patients with increased risk of death and treatment should be tailored in order to decrease the mortality rate.

Some limitations for our study including: This study was a retrospective research thereby diminished its power. An randomized controlled cohort study should be conducted in the future to further confirm our findings; Secondly, our sample size was relatively small; Next, some other parameters that might affect oxygenation index were not included in this study such as blood pressure.

Conclusion
Predicting prognosis of patients with pneumonia is critical for clinical doctors. Some biomarkers have
been well accepted in clinic to adjust treatment strategies to avoid bad ending. APACHE II score, LIS, SOFA, Nutric scores, WBC, neutrophils, lymphocyte counts, RAGE and albumin levels were independent risk factors for severe pneumonia with hypoxemia. RAGE is rarely used as biomarker. However, our study suggested that it had a predictive value for severe injury in pneumonia. While LIS, SOFA, lactate, lymphocyte, platelet, BUN, total bilirubin, PCT, and OSM levels before treatment were independent factors that were associated with 30 day-death. We discovered that OSM level at RICU admission was associated with 30 day-death. Furthermore, we discovered that if lactic acid, WBC, neutrophils, lymphocyte, erythrocyte, hemoglobin, hematocrit, neutrophils/lymphocyte, platelet, creatinine, total bilirubin, PT, APTT, Fib, PCT, RAGE, OSM and albumin levels had not been improved after one week treatment, patients were associated with increased mortality rate.

Abbreviations
RICU: respiratory intensive care unit; APACHE II: acute physiology, age, chronic health evaluation; LIS: lung injury score; SOFA: sequential organ failure assessment; NUTRIC score: The Nutrition Risk in Critically ill score; PCT: procalcitonin; CRP: C-reactive protein; ARDS: acute respiratory distress syndrome; CD4: CD4+T cell; CD8:CD8+T cell; CD3:CD3+T cell; PH: potential of hydrogen; PaCO2, partial pressure of arterial carbon dioxide; Lac, lactic acid; WBC, white blood cell count; BUN, blood urea nitrogen; LDH, lactate dehydrogenase; PT, prothrombin time; APTT, activated partial thromboplastin time; FiB, fibrinogen; RAGE: the receptor for advanced glycation end products; OSM: Oncostatin M; AUC, area under the curve; CI, confidence interval; ROC, receiver operating characteristic.

Declarations
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Author Contributions: Dr. Li Wang had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Yi Shi, Yan Tan were responsible for the study concept and design. JiangNan Zhao, Lin Gao and Jing Lei were responsible
for the data collection and analysis. All authors read and approved the final manuscript.

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**Availability of data and materials**

All data generated or analyzed during this study are included in this published article.

**Ethics approval and consent to participate**

Informed consent was obtained from patients’ legal representatives. The protocol was approved by the ethics committee of Jinling Hospital and Nanjing First Hospital (Approval Number: JLYY: 2013021).

**Consent for publication:** Not applicable.

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Figures
Figure 1

In order to examine the relationship between oxygenation index and the RAGE, we calculated the Pearson Correlation Coefficient with -0.228, P=0.001(Figure 1), indicating a meaningful negative correlation between RAGE and oxygenation index.
Figure 2

OSM level before treatment was associated with the pneumonia mortality. The Pearson correlation coefficient was -0.228, and $P=0.001$. 

Figure 2
The AUC of BUN before treatment was calculated to be 0.738 (95% CI: 0.607–0.869, P=0.002) with a cutoff point of 9.22mmol/L yielding a sensitivity and specificity of 81.3% and 64.3%, respectively (Figure 3).
Figure 4

ROC curves of serum total bilirubin and platelet resulted in an AUC of 0.69 (95% CI: 0.557-0.824, P=0.014) and 0.287 (95% CI: 0.127-0.447, P=0.006), respectively, using a cutoff value of 8.55 umol/L for serum total bilirubin with a sensitivity of 93.8% and specificity of 40.9%, and a cutoff value of 28(×10^9/L) for platelet with a sensitivity and specificity of 100% and 0% (Figure 4).
In order to identify the risk factors for death, we calculated the AUC of CRP and PCT after treatment which were 0.868 (95% CI: 0.712-1.000, P=0.003) and 0.855 (95% CI: 0.733-0.977, P=0.004) with cutoff values being 70.15mg/L and 0.24ng/ml respectively. The sensitivity/specificity was 83.3%/87.9% and 100%/72.4% respectively. (Figure 5).