Serum S100A8 as an early diagnostic biomarker in patients with community-acquired pneumonia

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Abstract

Background and Objectives

Limited studies suggested that calprotectin may take part in the pathophysiology of community-acquired pneumonia (CAP). Nevertheless, there is no clinical study to analyze the role of S100A8 in CAP patients. The objective of this study was to analyze the association of serum S100A8 with the severity of CAP based on a cross-sectional study.

Methods

Entire 200 CAP patients and 100 normal subjects were recruited. Demographic data, clinical information and serum were collected on admission. Serum S100A8 and inflammatory cytokines were detected.

Results

Serum S100A8 was increased in CAP patients on admission. Serum S100A8 was gradually increased in parallel with the CAP severity scores. Serum S100A8 was positively correlated with CAP severity scores (CURB-65, CRB-65, PSI, CURXO and SMART-COP), blood routine parameters (WBC, neutrophil-lymphocyte ratio and monocyte-lymphocyte ratio) and inflammatory cytokines (TNF α , IL-1 β and CRP). Furtherly, univariate and multivariate logistical regression analysis revealed that there was a positive association between serum S100A8 with CRB-65, PSI and CURXO. Moreover, the predictive capacity of serum S100A8 was performed by receiver operating characteristic area under the curve (AUC) analysis. The AUCs of S100A8 for CAP and CAP severity were 0.855 and 0.893, respectively. Mechanistic analysis found that S100A8 knockdown alleviated streptococcus pneumoniae-evoked inflammatory cytokines in A549 cells.

Conclusion

Serum S100A8 on admission was positively associated with the severity of CAP. S100A8 knockdown alleviates streptococcus pneumoniae-evoked inflammatory cytokines in A549 cells, indicating that S100A8 may exert an important role in the pathophysiology of CAP and be an early serum diagnostic biomarker for CAP.

Introduction

Community acquired pneumonia (CAP) is an infectious disease caused by bacteria, viruses, or a combination of these infectious agents [1]. This disease is popular among cases aged 50–60 years and under 5 years, always emerges when it is cold, especially in winter and early spring [2]. Now, CAP is increasingly common worldwide and responsible for significant morbidity and mortality [3]. Assessment of severity and the risk for pneumonia at the time of initial diagnosis are necessary for optimal CAP management, including selection of the best site of care (outpatient, inpatient general floor, or ICU) [4,5]. However, early severity assessment and risk stratification for CAP are challenging because obvious clinical characteristics at early stage are not highly predictive of which patients will suffer from

deterioration of their condition. The severity of the clinical manifestations in CAP patients significantly varies [6]. CAP has become a vital growing public health issue and medical curiosities, it elevates emotional and financial pressure to family, society and the government [7]. Therefore, it is beneficial to seek for biomarkers to diagnose, predict the severity and identify ways to reduce the incidence or severity.

Calprotectin (S100A8 and S100A9), are Ca^{2+} binding proteins belonging to the S100 family, which are expressed in a wide variety of cell types and abundant in myeloid cells, such as: neutrophils, monocytes, keratinocytes and early differentiation states of macrophages [8,9]. S100A8 and S100A9 form non-covalently associated complexes that exhibit typical properties of damage-associated molecular patterns (DAMPs), which are released by activated granulocytes and act in a cytokine-like manner through binding to cell surface receptors, such as toll-like receptor 4 (TLR4), scavenger receptor CD36 or receptor of advanced glycation end products (RAGE), which triggers signaling pathways involved in the inflammatory processes and plays critical roles in numerous cellular processes [10–13]. Several studies found that S100A8 was increased in such inflammatory diseases including: inflammatory bowel disease, chronic obstructive pulmonary disease, rheumatoid arthritis, cystic fibrosis, autoimmune disease and neurodegenerative disorders [14–18]. It is suggested that S100A8 can be considered as a significant biomarker for diagnostic purposes.

An earlier animal experiment found that high expression of S100A8 protein in the pulmonary alveolar walls in mice infected with *streptococcus pneumoniae* [19]. In vitro experiment demonstrated that S100A8 protein was increased in the bronchial epithelium after inflammation stimulation [20]. In addition, S100A8 was highly abundant and secreted into lung lavage fluid in mice after lipopolysaccharide exposure [21]. These data indicated that S100A8 may exert important role in the infectious diseases. However, the role of S100A8 protein in CAP is still unclear. Therefore, we speculate that S100A8 heterodimer may take part in the pathogenesis of CAP. Nevertheless, there is no clinical and experiment research demonstrating the role of S100A8 heterodimer in CAP. Hence, the main goal of this study was to explore the correlations between serum S100A8 heterodimer with the severity of CAP and inflammatory cytokines in CAP patients with a population-based retrospective cross-sectional study.

Materials And Methods

Subjects

The Second Affiliated Hospital of Anhui Medical University is a tertiary care university hospital in Hefei City, Anhui Province, China. This retrospective study was performed in the Department of Respiratory and Critical Care Medicine from May 2018 to May 2020. This study was approved by the Ethics Committee in Second Affiliated Hospital of Anhui Medical University. In total, 200 CAP patients (112 males and 88 females) and 100 healthy subjects were recruited in this study. Demographic and clinical information were collected of CAP patients on admission. All CAP patients gave advanced written and oral agreement of their inclusion and signed a consent form in this study. For CAP patients, the inclusion criteria consisted of inpatients more than 30 years old, who were diagnosed and admitted for treatment of CAP in

the intensive care units or general wards. Exclusion criteria included being an outpatient; with other pulmonary diseases, serious complications, tuberculosis, malignancy and asthma; having an organ or bone marrow transplant; being severely immunocompromised. All CAP patients were given empirical antimicrobial agents intravenously within the first 48 h. Thereafter, antibiotics with either orally or intravenously were given based on established guidelines. Pneumonia severity was evaluated by the CAP severity score, including pneumonia severity index (PSI), CURB-65 score, CRB-65 score, CURXO score and SMART-COP score [22]. Blood samples were collected on admission before treatment. For the healthily normal subjects, 100 healthily normal subjects were randomly enrolled from the physical examination center in the Second Affiliated Hospital of Anhui Medical University. Following fundamental data were collected from the electronic medical records of CAP patients and healthy subjects: demographic information, preexisting comorbidities, symptoms and signs of CAP, laboratory examination data.

Cell culture

Human carcinoma lung epithelial (A549) cell line was from American Type Culture Collection (USA). A549 cells were cultured with RPMI 1640 medium (HyClone; Logan, UT) supplemented with 7.5% fetal bovine serum (FBS), 100 U/ml penicillin and 100 mg/ml streptomycin in a humidified chamber at 37 °C with 5% CO₂. *Streptococcus pneumoniae* was used for stimulations at a concentration of 10⁷ CFU/mL based on a previous study [23]. A549 cells were seeded into 25-cm² culture dishes. When the cells were cultured to 50% density, all cells were incubated with *streptococcus pneumoniae* for 12 h. Cell supernatant were collected for enzyme-linked immunosorbent assay (ELISA). After 12 h, the cells were washed with chilled PBS three times and then harvested for real-time RT-PCR.

Small interfering RNA (siRNA) transfection

Human S100A8 and scrambled siRNAs were obtained from Gene Pharma Corp (Shanghai, China). The S100A8 siRNA sequences was as follows: (sense, 5'- GGUCACUACUGAGUGCCCUCAGUUU- 3'; antisense, 5'- AAACUGAGGGCACUCAGUAGUGACC- 3'); The RNA interference protocol was on the basis of the previous study [24]. Human S100A8-siRNA was transfected into cells using Lipofectamine 3000 according to the manufacturer's instructions (Invitrogen, Carlsbad, CA, United States). After 4 h incubation, the medium was replaced with fresh RPMI-1640 and the cells were incubated for another 48 h. After siRNA transfection for 48 h, *streptococcus pneumoniae* was continued to co-culture and A549 cells were harvested.

Isolation of total RNA and real-time RT-PCR

Total RNA from human lung epithelial cells were extracted using TRI reagent followed by a cleaning up step [25]. The purified RNA was transcribed into cDNA using avian myeloid leukemia virus reverse transcriptase (Promega) and analyzed by real-time RT-PCR with a Light Cycler 480 SYBR Green I kit (Roche Diagnostics). The gene-specific primers were shown in Supplemental Table 1. The levels of

mRNAs were calculated using the standard curve method and expressed as fold of control expression. Rn18S expression was used for the normalization of each protein expression levels for all samples.

Enzyme-linked immunosorbent assay (ELISA)

S100A8 ELISA kits were prepared from Wuhan ColorfulGene Biological Technology Co., Ltd. CRP, TNF- α , IL-1 β and IL-6 ELISA kits were purchased from Cusabio, Wuhan, China (<https://www.cusabio.com/>). All cytokines were detected on the basis of the manufacturer's instructions [26].

Statistical analysis

All statistical analyses were performed using SPSS 19.0. Student's *t* tests, Chi-square tests, and Mann-Whitney U tests were used to compare the demographic characteristics of means, proportions and medians, respectively. Linear regression analyses were used to examine the associations between S100A8 and pneumonia severity, inflammatory cytokines and blood routine test. Moreover, logistical regression analysis was performed between serum S100A8 and CAP severity score. Categorical variables were expressed with frequencies and percentages. Continuous variables were shown using median and mean values. Statistical significance was determined at $P \leq 0.05$.

Results

Demographic and clinical information

Altogether 200 CAP patients and 100 normal subjects were enrolled in the Second Affiliated Hospital of Anhui Medical University. The demographic and clinical information were analyzed between CAP patients and normal subjects. As shown in Table 1, no obvious difference of age, sex, BMI, systolic pressure and diastolic pressure was observed among two groups. Moreover, the cases of comorbidities with hypertension, diabetes mellitus, cancer, interstitial pneumonia, chronic obstructive pulmonary disease, cor pulmonale, chronic kidney disease and neurological disease were more in CAP patients than those in normal subjects. There was no difference of chronic liver disease, cardiovascular disease and other diseases between two groups. Eighteen (9.0%) CAP patients were died after hospitalization. The average hospital stay was 10.0 days. In addition, pneumonia severity was evaluated using CAP severity score. Of 200 CAP patients, 82 (41.0%) severe patients were in the CAP cases (CURXO score), the median PSI score, CURB-65 score, CRB-65 score and SMART-COP score was 2.0, 1.0, 92.0 and 2.0, respectively.

The levels of serum S100A8 heterodimer in control subjects and CAP patients

Serum S100A8 heterodimer was measured in CAP patients and control subjects. As shown in Figure 1A, serum S100A8 was obviously increased in CAP patients compared with those in control subjects (69.23 pg/mL vs 163.32 pg/mL). Besides, serum S100A8 heterodimer was analyzed among different grade of CAP patients. As shown in Figure 1B, serum S100A8 heterodimer was increased in 0 score grade than those in 1–2 score grade and ≥ 3 score grade based on CRB-65 score. Serum S100A8 was higher in ≥ 3 score grade than those in 1–2 score grade. According to CURB-65 score, serum S100A8 heterodimer was

gradually increased in parallel with the CURB-65 score (Figure 1C). Additionally, we found that serum S100A8 heterodimer was higher in severe CAP patients than those in mild CAP patients (CURXO score) (Figure 1D). Besides, on the basis of SMART-COP score, serum S100A8 heterodimer in 0-2 score grade was lowest and in 7-8 score grade was highest, serum S100A8 heterodimer in 5-6 score grade was higher than those in 0-2 score grade (Figure 1E). In addition, serum S100A8 heterodimer was compared among different grades of CAP patients based on PSI score. As shown in Figure 1F, serum S100A8 heterodimer was gradually elevated parallelly with the PSI score.

Associations of S100A8 heterodimer with disease severity, blood routine parameters and inflammatory cytokines of CAP patients

The association between S100A8 heterodimer and disease severity was analyzed among CAP patients. As shown in Table 2, serum S100A8 heterodimer was positively and significantly associated with CURB-65 ($r = 0.541$, $P = 0.001$), CRB-65 ($r = 0.598$, $P = 0.001$), PSI ($r = 0.611$, $P = 0.001$), CURXO ($r = 0.546$, $P = 0.001$) and SMART-COP ($r = 0.492$, $P = 0.001$). Moreover, the associations between serum S100A8 heterodimer and blood routine parameters were evaluated. There were no obvious associations between serum S100A8 with neutrophil and PLR (platelet-lymphocyte ratio). Serum S100A8 heterodimer was weakly and positively associated with WBC (white blood cell) ($r = 0.294$, $P = 0.003$), NLR (neutrophil-lymphocyte ratio) ($r = 0.319$, $P = 0.001$) and MON (monocyte-lymphocyte ratio) ($r = 0.223$, $P = 0.027$). Additionally, the associations of serum S100A8 and inflammatory cytokines were calculated. As shown in Table 2, serum S100A8 was positively and significantly associated with TNF α ($r = 0.396$, $P = 0.005$), IL-1 β ($r = 0.310$, $P = 0.001$) and CRP ($r = 0.345$, $P = 0.027$). There was no significant association between serum S100A8 and IL-6 ($r = 0.398$, $P = 0.055$). Furthermore, associations of serum S100A8 with CAP severity indices were analyzed with univariate logistic regression among CAP patients. As shown in Table 3, serum S100A8 level was evidently and positively with CURB-65 ($\beta = 1.286$; 95% CI: 1.050, 1.786), CRB-65 ($\beta = 1.345$; 95% CI: 1.004, 2.415), PSI ($\beta = 1.245$; 95% CI: 1.009, 1.894), SMART-COP ($\beta = 1.286$; 95% CI: 1.050, 1.786) and CURXO ($\beta = 1.018$; 95% CI: 1.007, 1.030). In order to control confounders, associations of serum S100A8 with CAP severity score were furtherly analyzed with multivariate logistic regression. Although, there was no obvious association between serum S100A8 level with CURB-65 and SMART-COP after adjustment for confounders (such as age, sex, BMI and comorbidities). We found that serum S100A8 level was significantly and positively correlated with CRB-65 ($\beta = 1.112$; 95% CI: 1.001, 1.421), PSI ($\beta = 1.121$; 95% CI: 1.004, 1.415) and CURXO ($\beta = 1.118$; 95% CI: 1.012, 1.321) (Table 3).

ROC curves and cut-off point analysis for serum S100A8

Based on the data shown so far, an evaluation of the predictive capacity of serum S100A8 was performed by receiver operating characteristic area under the curve (AUC) analysis. As shown in Figure 2A, the AUC of serum S100A8 for the prediction of CAP was 0.855 (95% CI: 0.791, 0.919). A numerical threshold set at 86.89 pg/mL to minimize the risk of false-negative diagnosis allowed the identification of CAP with a 74% sensitivity and specificity (87%). Moreover, the AUC of serum S100A8 for the prediction of severity was analyzed among CAP patients. As shown in Figure 2B, the AUCs were as follows: S100A8,

0.893 (95% CI: 0.743, 0.904); CURB-65, 0.894 (95% CI: 0.832, 0.954); CRB-65, 0.886 (95% CI: 0.823, 0.950); PSI, 0.939 (95% CI: 0.891, 0.987); SMART-COP, 0.965 (95% CI: 0.932, 0.998); CURXO, 0.880 (0.810, 0.851). In addition, the optimal cutoff value of S100A8 for CAP was 158.32 pg/mL, with a specificity of 78% and sensitivity of 82% (Figure 2B).

S100A8 knockdown alleviated *streptococcus pneumoniae* infection-evoked inflammatory cytokines in human pulmonary epithelial cells

To further explore the possible mechanism of S100A8 elevation in CAP patients, A549 cells were transfected with *S100A8* siRNA and then exposed to *streptococcus pneumoniae* to observe whether *S100A8* knockdown attenuates *streptococcus pneumoniae* injection-evoked inflammatory cytokines in human pulmonary epithelial cells. As expected, transfection with *S100A8* siRNA obviously decreased *S100A8* mRNA in A549 cells (Figure 3A). These results indicated that *S100A8* siRNA transfection significantly and effectively knockdown S100A8 expression in A549 cells. Next, S100A8 and inflammatory cytokines were determined after *streptococcus pneumoniae* exposure in A549 cells. *S100A8* mRNA and supernatant S100A8 level were all increased after *streptococcus pneumoniae* exposure in A549 cells (Figure 3A, 3B). Interestingly, transfection with S100A8 siRNA obviously alleviated *streptococcus pneumoniae*-induced upregulation of *S100A8* mRNA in A549 cells (Figure 3A, 3B). Moreover, mRNAs of inflammatory cytokines (*IL-1 β* , *CRP*, *TNF α* and *IL-6*) were elevated in A549 cells after *streptococcus pneumoniae* injection (Figure 3C-3F). Even more impressively, pretreatment with *S100A8* siRNA transfection dramatically mitigated *streptococcus pneumoniae* injection-induced elevation of inflammatory cytokines (Figure 3C-3F).

Discussion

As far as we know, this was the first epidemiological and laboratory study to investigate the association of serum S100A8 and the severity among CAP patients. The major findings of this study include: (1) Serum S100A8 heterodimer is increased in CAP patients on admission; (2) Serum S100A8 heterodimer is higher in severe CAP patients than in mild CAP patients on admission; (3) Serum S100A8 heterodimer is positively associated with CAP severity indices on admission; (4) *S100A8* knockdown attenuates *streptococcus pneumoniae* injection-evoked inflammatory cytokines in human lung epithelial cells.

Previous in vivo experiment has found that S100A8 was highly expressed in the pulmonary alveolar walls in mice infected with *streptococcus pneumoniae* [19]. In vitro experiment has demonstrated that S100A8 protein was increased in the bronchial epithelium after inflammation exposure [20]. In addition, S100A8 was secreted into lung lavage fluid in mice after lipopolysaccharide stimulation [21]. However, there are limited clinical studies exploring the role of S100A8 in CAP patients. In the present research, we found that serum S100A8 heterodimer was increased in CAP patients compared with control subjects. According to the CAP severity indices, serum S100A8 heterodimer gradually increased in parallel with the severity of CAP. Besides, there were positive correlations between serum S100A8 heterodimer and the CAP severity scores. Logistical regression analysis further confirmed that serum S100A8 heterodimer was

positively associated with CRB-65 score, PSI score and CURXO score. These results indicate that serum S100A8 heterodimer was positively associated with the severity of CAP.

More and more studies have found that inflammation reaction participates in the pathogenesis of CAP patients. C-reactive protein (CRP) and many pro-inflammatory cytokines (IL-1 β , TNF α and IL-6) were increased in CAP patients [27–29]. Inflammatory cytokines were positively associated with the severity among CAP patients [30]. Serum S100A8 was positively associated with several pro-inflammatory cytokines in inflammatory diseases [30,31]. However, the associations between serum S100A8 and inflammatory cytokines remained unclear among CAP patients. The present study analyzed the associations of serum S100A8 with inflammatory cytokines in CAP patients. These results indicated that serum S100A8 heterodimer was positively associated with TNF α , IL-1 β and CRP. Moreover, a study found that blood routine parameters can be as indicators for CAP [32]. This study analyzed the correlations between serum S100A8 heterodimer and blood routine parameters in CAP patients. We found that there was a weakly positive correlation between serum S100A8 heterodimer with white blood cell, neutrophil-lymphocyte ratio and monocyte-lymphocyte ratio. These results suggest serum S100A8 heterodimer could be a diagnostic biomarker for CAP.

A productive relationship of serum S100A8 heterodimer with the CAP severity score indicated that it may be believed as a potential predictive biomarker in CAP patients. In the present research, we analyzed the predictive power through performing a sensitivity/specificity analysis with ROC curve test. The AUC values always represent the predictive quality. The optimal cutoff value of S100A8 for CAP was 0.225, with a specificity of 81.6% and sensitivity of 82.5%. Moreover, we analyzed the predictive power for CAP severity S100A8 and the CAP severity score. The results demonstrated that S100A8 yielded higher AUC values than CRB-65 and CURXO scores, was similar with CURB-65 score. Furthermore, we found that the predictive capacity of S100A8 is better than many known biomarkers though literature review [32,33]. Serum S100A8 is more easily to detected and obtained than pneumonia severity scores in same cases. Serum S100A8 heterodimer may have more advantage over CAP severity score of diagnosing CAP. Therefore, these results imply that serum S100A8 heterodimer can be used as a better diagnostic biomarker in CAP patients.

Despite constant efforts to develop the association of S100A8 with CAP and to ascertain the molecular mechanism leading to the pathophysiology of CAP, much remains unknown. Mounting evidence suggests S100A8 exhibits typical properties of DAMPs, which is released through activating granulocytes, acts in a cytokine-like manner through binding to cell surface receptors, such as TLR4, CD36 or RAGE. S100A8 can activate several inflammatory signaling pathways and plays critical roles in numerous cellular processes [10–13]. During inflammatory environment, S100A8 is released and modulates the inflammatory response through stimulating leukocyte recruitment and evoking cytokine secretion. The release of S100A8 can elevate the multiple cytokines in inflammatory cells to sustain and exacerbate inflammation [34,35]. An earlier research found that S100A8 release induced MyD88 translocation and activated NF- κ B signaling, resulting in TNF α secretion in phagocytes [36]. Elevated inflammatory host responses results in the unfavorable outcome by driving lung failure and pneumonia. Moreover, our results found that *S100A8*

knockdown alleviated *streptococcus pneumoniae* injection-evoked inflammatory cytokines in human pulmonary epithelial cells. So, we speculate that *streptococcus pneumoniae* injection induces CAP, partially through S100A8-mediated inflammatory reaction.

In brief, this research finds that S100A8 may take part in the development and progression of CAP. These findings may further promote better understanding the pathogenesis of, and to seek for the potential diagnostic biomarkers for CAP. Nevertheless, there are some flaws in this study. Firstly, this was only a cross-sectional study and in vitro experiment, the causal link between S100A8 heterodimer and CAP patients is needed to demonstrate using further longitudinal studies and animal experiments in the future research. Secondly, this was a single-center and small-sample study, a larger sample size from multicenter survey is needed in the next work. Thirdly, the mechanism of S100A8-aggravated the severity of CAP was not clear, more laboratory researches are needed to perform in the future. Fourthly, S100A8 heterodimer was only measured in the serum, the levels of S100A8 in sputum and bronchoalveolar lavage fluid are uncharted among CAP patients.

Conclusion

To summarize, serum S100A8 heterodimer is increased in CAP patients at early stage. Higher serum S100A8 heterodimer is positively correlated with the severity of CAP at early stage. *S100A8* knockdown alleviates *streptococcus pneumoniae*-evoked inflammatory cytokines in the pulmonary epithelial cells, indicating that S100A8 heterodimer may take part in the development and progression of CAP. Consequently, S100A8 heterodimer can be used as an early serum diagnostic biomarker and potentially therapeutic target for CAP in the future clinical practice.

Declarations

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Authors' contributions

Fu L and Zhao H conceived the study; Fu L, Zhao H, Zheng L and Fei J participated in the design of this study; Zheng L, Fei J, Xu Z, Feng CM, Li SR and Xu DX conducted the research; Fu L conducted statistical analyses of all data and drafted the manuscript. All authors read and approved the final manuscript.

Conflict of interest

All authors have declared that no competing interest exists.

Ethical Approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Availability of data and materials

The datasets generated and analyzed during the current study are not publicly available due to identifiable information of patients but are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

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Abbreviations

CAP, community-acquired pneumonia; WBC, white blood cell; NLR; neutrophil-lymphocyte ratio; MON, monocyte-lymphocyte ratio; PLR, platelet-lymphocyte ratio; TNF α , tumor necrosis factor α ; IL-1 β , interleukin-1 β ; IL-6, interleukin-6; CRP, C-reactive protein; Receiver operating characteristic, ROC; Area under the curve, AUC.

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Variables	CAP (n=200)	Control (n=100)	<i>P</i>
Age (years)	66.5 (56.5, 78.0)	62.0 (51.6, 73.8)	0.452
Male, n (%)	112 (56.0)	108 (54.0)	0.321
BMI	22.6 (20.1, 25.6)	21.3 (19.2, 24.3)	0.221
Systolic pressure (mmHg)	122.5 (108.0, 135.0)	120.1 (103.5, 139.5)	0.564
Diastolic pressure (mmHg)	73.0 (66.3, 80.8)	78.0 (60.5, 84.6)	0.521
Comorbidities			
Hypertension, n (%)	44 (22.0)	9 (9.0)	0.005
Diabetes mellitus, n (%)	24 (12.0)	0	0.001
Cancer, n (%)	25 (12.5)	0	0.001
Interstitial pneumonia, n (%)	18 (9.0)	0	0.002
COPD, n (%)	12 (6.0)	0	0.012
Cor pulmonale, n (%)	16 (8.0)	0	0.004
Chronic liver disease, n (%)	20 (10.0)	4 (4.0)	0.071
Chronic heart disease, n (%)	6 (3.0)	3 (3.0)	1.000
Chronic kidney disease, n (%)	8 (4.0)	0	0.043
Cardiovascular disease, n (%)	21 (10.5)	5 (5.0)	0.110
Neurological disease, n (%)	10 (5)	0	0.023
Other disease, n (%)	31 (15.5)	12 (12.0)	0.415
Hospital stay (day)	10.0 (7.0, 17.0)	N.A	N.A
Mortality, n (%)	18 (9.0)	N.A	N.A
CURB-65	2.0 (0, 3.0)	N.A	N.A
CRB-65	1.0 (0, 2.0)	N.A	N.A
PSI	92.0 (58.0, 128.0)	N.A	N.A
CURXO [Severe, n (%)]	82 (41.0)	N.A	N.A
SMART-COP	2.0 (0, 5.0)	N.A	N.A

Table 2. Correlations between serum S100A8 with disease severity, blood routine and inflammatory cytokines.

Severity scoring systems					
Variables	CURB-65	CRB-65	PSI	CURXO	SMART-COP
<i>r</i>	0.541	0.598	0.611	0.546	0.492
<i>P</i>	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001
Blood routine parameters					
Variables	WBC	Neutrophil	NLR	MON	PLR
<i>r</i>	0.294	0.156	0.319	0.223	-0.028
<i>P</i>	0.003	0.126	≤0.001	0.027	0.784
Inflammatory cytokines					
Variables	TNFα	IL-1β	CRP	IL-6	
<i>r</i>	0.396	0.310	0.345	0.398	
<i>P</i>	0.005	≤0.001	0.002	0.055	

Table 3. Associations between serum S100A8 with CAP severity scores among CAP patients.

Variables	Univariable (95% CI)	<i>P</i>	Multivariable (95% CI)*	<i>P</i>
CURB-65	1.286 (1.050, 1.786)	0.003	1.007 (0.993, 1.022)	0.312
CRB-65	1.345 (1.004, 2.415)	0.001	1.112 (1.001, 1.421)	0.040
PSI	1.245 (1.009, 1.894)	0.001	1.121 (1.004, 1.415)	0.031
SMART-COP	1.286 (1.050, 1.786)	0.003	1.017 (0.998, 1.035)	0.085
CURXO	1.018 (1.007, 1.030)	0.001	1.118 (1.012, 1.321)	0.038

Dependent variables: S100A8. Independent variable: CURB-65, CRB-65, PSI, SMART-COP, CURXO.

*Adjusted for age, sex, BMI and comorbidities.

Figures

Figure 1

Serum S100A8 levels between CAP patients and control subjects. (A) Serum S100A8 levels in CAP patients and normal subjects (n=100 for normal subjects; n=200 for CAP patients). (B-F) Serum S100A8 levels in different grades of CAP severity. (B) CRB-65 score. (C) CURB-65 score. (D) CURXO score. (E) SMART-COP score. (F) PSI score. All data were represented as mean $\pm$ SEM. * $P \leq 0.05$, ** $P \leq 0.01$.

Figure 2

Receiver operating characteristic curves for different predictive biomarkers on admission. (A) ROC curve was used to evaluate the diagnostic value of serum S100A8 for CAP. (B) ROC curve was used to evaluate the diagnostic value of different predictive biomarkers (S100A8, CRB-65, CURB-65, CURXO, SMART-COP and PSI) for the severity of CAP.

Figure 3

The levels of S100A8 and inflammatory cytokines after streptococcus pneumoniae injection in A549 cells (A) S100A8 mRNA was detected using RT-PCR after streptococcus pneumoniae (S.P.) injection in A549 cells. (B) Supernatant S100A8 level was measured using ELISA after S.P. injection in A549 cells. (C-F) The mRNAs of inflammatory cytokines were detected using RT-PCR after S.P. injection in A549 cells. (C) IL-1 β . (D) CRP. (E) TNF α . (F) IL-6. All data were represented as mean $\pm$ SEM of six samples (n = 6). *P $\leq$ 0.05, **P $\leq$ 0.01.

Supplementary Files

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