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*Original Article*

# Prognostic value of IL-6, HMGB1, and TLR4 in bronchoalveolar lavage fluid of patients with community-acquired pneumonia

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**Abstract:** Objectives: To evaluate the prognostic value of inflammatory biomarkers in bronchoalveolar lavage fluid (BALF) in patients with community-acquired pneumonia (CAP). Methods: A retrospective case-control study was conducted on 109 patients with CAP who were admitted between June 2022 and May 2024. Based on clinical outcome on day 10 after treatment initiation, patients were classified into an improved group (n = 58) and a deteriorated group (n = 51). Levels of white blood cells (WBC), C-reactive protein (CRP), interleukin-6 (IL-6), high-mobility group box 1 (HMGB1), and Toll-like receptor 4 (TLR4) in BALF were measured and compared between the two groups. Risk factors associated with poor prognosis were identified using logistic regression analyses, and a predictive model for disease deterioration was developed incorporating the identified independent risk factors. The clinical use of the predictive model was evaluated using receiver operating characteristic (ROC) analysis and decision curve analysis (DCA). Results: The deteriorated group had significantly higher levels of WBC, CRP, IL-6, HMGB1, and TLR4 (all $P < 0.05$). IL-6, HMGB1, and TLR4 showed strong positive correlations with poor prognosis (all $P < 0.001$). Multivariate logistic regression confirmed IL-6 (OR = 1.052, $P < 0.001$), HMGB1 (OR = 6.769, $P < 0.001$), and TLR4 (OR = 66.929, $P = 0.002$) as independent predictors of adverse outcome. A combined predictive model incorporating IL-6, HMGB1, and TLR4 demonstrated excellent discriminative ability, with an AUC of 0.940, outperforming each single marker. Conclusions: IL-6, HMGB1, and TLR4 in BALF are critical prognostic biomarkers in CAP, reflecting their roles in inflammatory response and disease progression.

**Keywords:** Community-acquired pneumonia, prognostic biomarkers, inflammatory response, IL-6, HMGB1, Toll-like receptor 4

## Introduction

Community-acquired pneumonia (CAP) remains a major global health concern, associated with substantial morbidity, mortality, and healthcare burden, particularly among the elderly and immunocompromised individuals [1, 2]. Despite advances in antibiotic and vaccine therapies, effective disease management and accurate prognostic prediction remain challenging. This difficulty arises from heterogeneity in causative pathogen, host immune response, and clinical presentation among patients [3, 4]. Consequently, identifying reliable biomarkers is crucial for early diagnosis, risk stratification, and prognostic assessment in CAP. Inflammatory biomarkers have attracted considerable attention for their ability to predict host immune responses and disease severity. Compared to circulating blood markers, biomarkers derived from bronchoalveolar lavage fluid (BALF) may provide a more direct and sensitive assessment of pulmonary inflammation and immune activation at the site of infection [5-7].

BALF, obtained from the distal airways and alveolar spaces, represents a unique biologic material distinct from serum or plasma. BALF directly captures host-pathogen interactions within the lung microenvironment and contains a wide range of bioactive molecules, including cytokines, chemokines, and other immune mediators, thereby offering valuable insight into local inflammatory processes in respiratory diseases [8-10]. Evidence indicates that inflamma-

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tory mediators play critical roles in the pathogenesis and progression of CAP, influencing infection severity and clinical outcome [11].

Previous studies have primarily focused on individual inflammatory markers, such as C-reactive protein (CRP), interleukins, tumor necrosis factor α (TNF-α), and procalcitonin [12, 13]. However, results across studies have been inconsistent, and growing attention has shifted toward comprehensive profiling of inflammatory biomarkers in BALF. Elucidating the complex interplay between these biomarkers may advance our understanding of the underlying immunopathologic mechanisms and facilitate the identification of clinically relevant prognostic indicators. A biomarkers panel derived from BALF may help stratify patients at higher risk of adverse outcome, enabling tailored therapeutic intervention [14, 15].

Technological innovations, particularly high-throughput multiplex assays, have enhanced the prognostic use of BALF-derived biomarkers by enabling simultaneous quantification of multiple analytes from limited sample volumes. This methodology represents a shift from conventional single-marker analyses to comprehensive profiling of the inflammatory milieu within the lung. Furthermore, integrating machine learning into biomarker research supports sophisticated analytic modeling, allowing identification of complex biomarker signatures and construction of multi-marker indices with improved predictive performance for clinical outcome [16-18].

This study aimed to identify prognostic biomarkers among inflammatory mediators in BALF from patients with CAP. By evaluating their association with disease progression, this study aimed to deepen understanding of CAP pathophysiology and clarify the roles of these biomarkers in clinical deterioration. The findings may contribute to improved early risk stratification, and support timely and targeted clinical intervention that reduces hospitalization duration and overall healthcare burden.

## Patients and methods

### *Study design*

This retrospective study included 109 patients with CAP treated at Jinhu County People's Hospital between June 2022 and May 2024. Demographic and clinical data were systematically collected, including baseline information, vital signs, Pneumonia Severity Index (PSI) score, inflammatory marker levels, and clinical manifestations. This study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Jinhu County People's Hospital. The requirement for informed consent was waived by the ethics committee due to the retrospective design, as the study involved anonymized clinical data and previously stored samples.

### *Inclusion and exclusion criteria and grouping standards*

Inclusion criteria: (1) Age between 18 and 80 years; (2) Diagnosis of CAP according to established guidelines for the diagnosis and treatment of adult CAP [19]; and (3) Availability of complete clinical data.

Exclusion criteria (1) Autoimmune disease; (2) Malignancy; (3) Chronic hepatic or renal dysfunction; (4) Chronic airway diseases, including chronic obstructive pulmonary disease (COPD) and asthma; (5) Long-term use of steroids, (6) Current smoking history; (7) Severe malnutrition, defined as a body mass index (BMI) < 18 $kg/m^2$; and (8) Contraindications to or intolerance to bronchoscopy.

Based on treatment outcomes assessed on day 10 after initiation of combination therapy, the patients were divided into an improved group (n = 58) and a deteriorated group (N = 51). The improved group included patients who achieved recovery or significant clinical improvement. Recovery was defined as normalization of clinical symptoms, signs, and laboratory and etiological indicators. Significant improvement was defined as marked clinical improvement with at least one marker not fully restored to normal. In contrast, patients whose condition failed to improve or worsened after 10 days of treatment were defined as deteriorated.

### *BALF sample collection*

As part of standard clinical management, BALF samples were obtained within 24 hours of hospital admission. An electronic bronchoscope (Olympus 1T260 model) was used for bron-

choalveolar lavage. BALF was aspirated under negative pressure, filtered through double-layer sterile gauze to remove mucus, and transferred into sterile tubes. Samples were centrifuged at 1000×g for 20 minutes, and the supernatants were aliquoted and stored at -20°C in the institutional biobank until analysis. For this retrospective analysis, previously stored BALF samples were retrieved for biomarker measurement.

*Data collection*

Patient demographic and clinical information was extracted from the hospital electronic medical record system, including age, sex, BMI, education level, employment status, marital status, place of residence, history of alcohol consumption, and comorbidities (e.g., hypertension, diabetes, and coronary artery disease).

Vital signs at admission were recorded, including body temperature, heart rate, respiratory rate, and oxygenation. The Pneumonia Severity Index (PSI) score was calculated for each patient at admission to assess disease severity and predict mortality risk. The PSI stratifies patients into five risk classes (I-V) based on a weighted scoring system incorporating demographic characteristics, comorbid conditions, physical examination findings, laboratory results, and radiographic findings [20]. Risk class I is determined using an initial screening algorithm that identifies low-risk patients without the need for point scoring. For the remaining patients, a point-based system is applied: class II corresponds to a total score ≤ 70 points, class III to 71-90 points, class IV to 91-130 points, and class V to > 130 points. Higher PSI classes indicate greater disease severity and increased risk of mortality.

Inflammatory marker measurement: BALF supernatants stored at -20°C following centrifugation (1000×g, 20 minutes) were used for inflammatory marker quantification. White blood cell (WBC) counts were determined using an automated hematology analyzer (SYSMEX XN-1000, Sysmex Corporation, Kobe, Japan). The levels of interleukin-6 (IL-6), high-mobility group box 1 (HMGB1), and Toll-like receptor 4 (TLR4) were measured using high-sensitivity ELISA Kits (IL-6: R&D Systems, Minneapolis, MN, USA; HMGB1: IBL International, Hamburg, Germany; TLR4: Thermo Fisher Scientific, Waltham, MA, USA). C-reactive protein (CRP), interleukin-8 (IL-8), and tumor necrosis factor-α (TNF-α) were also quantified using ELISA Kits (CRP: DRG Instruments GmbH, Marburg, Germany; IL-8: R&D Systems; TNF-α: Thermo Fisher Scientific).

Clinical manifestations were recorded in detail at admission, including fever (body temperature ≥ 38°C), cough, sputum production, dyspnea, chest pain, fatigue, and altered consciousness.

*Statistical analysis*

Statistical analyses were performed using SPSS 29.0. Categorical data were presented as n (%) and compared using the Chi-square ($\chi^2$) test when the sample size was ≥ 40 and the theoretical frequency was ≥ 5. Yates' continuity correction was applied when the theoretical frequency was 1-5, and Fisher's exact test was used when expected frequencies were < 5. Continuous variables were tested for normality using the Shapiro-Wilk test. Normally distributed continuous data were presented as mean ± standard deviation (SD) and compared using the independent-samples t-test. Univariate and multivariate logistic regression analyses were performed to identify the independent risk factors for disease deterioration with odds ratios (ORs) and 95% confidence intervals (CIs) calculated. Correlations between variables were evaluated using Spearman's rank correlation coefficient. A predictive model for prognostic prediction was constructed based on identified risk factors. Receiver Operating Characteristic (ROC) curves were plotted to assess the discriminative performance of predictive models. Decision curve analysis (DCA) was conducted to evaluate the clinical value of the models. A *P*-value < 0.05 was considered significant.

## Results

*Baseline characteristics*

There were no significant differences between the two groups in terms of age, BMI, sex distribution, educational level, employment status, marital status, place of residence, history of alcohol consumption, or comorbidities (all P > 0.05; **Table 1**).

**Table 1.** Comparison of baseline characteristics between the two groups

| Item | Improved Group (n = 58) | Deteriorated Group (n = 51) | $t/\chi^2$ | P |
|---|---|---|---|---|
| Age (years) | 53.24 ± 10.21 | 54.18 ± 11.32 | 0.455 | 0.650 |
| BMI ($kg/m^2$) | 23.56 ± 2.12 | 23.84 ± 2.35 | 0.664 | 0.508 |
| Sex (Male/Female) | 34/24 | 29/22 | 0.034 | 0.853 |
| Educational Level [n (%)] | | | 0.106 | 0.948 |
| Primary or Below | 10 (17.24) | 8 (15.69) | | |
| Secondary School | 28 (48.28) | 24 (47.06) | | |
| University or Above | 20 (34.48) | 19 (37.25) | | |
| Employment Status [n (%)] | | | 0.015 | 0.993 |
| Employed | 32 (55.17) | 28 (54.90) | | |
| Unemployed | 12 (20.69) | 11 (21.57) | | |
| Retired | 14 (24.14) | 12 (23.53) | | |
| Marital Status [n (%)] | | | 0.022 | 0.989 |
| Married | 45 (77.59) | 40 (78.43) | | |
| Single | 8 (13.79) | 7 (13.73) | | |
| Divorced | 5 (8.62) | 4 (7.84) | | |
| Residence [n (%)] | | | 0.005 | 0.942 |
| Urban | 36 (62.07) | 32 (62.75) | | |
| Rural | 22 (37.93) | 19 (37.25) | | |
| Alcohol consumption history [n (%)] | 12 (20.69) | 11 (21.57) | 0.013 | 0.911 |
| Comorbidities [n (%)] | | | | |
| Hypertension | 16 (27.59) | 15 (29.41) | 0.044 | 0.833 |
| Diabetes Mellitus | 10 (17.24) | 9 (17.65) | 0.003 | 0.956 |
| Coronary Artery Disease | 8 (13.79) | 7 (13.73) | 0.000 | 0.992 |

Note: BMI: Body Mass Index.

**Table 2.** Comparison of baseline vital signs and psi scores between the two groups

| Item | Improved Group (n = 58) | Deteriorated Group (n = 51) | t | P |
|---|---|---|---|---|
| Temperature (°C) | 37.42 ± 0.53 | 37.56 ± 0.58 | 1.326 | 0.188 |
| Heart Rate (bpm) | 93.31 ± 12.45 | 95.61 ± 13.22 | 0.935 | 0.352 |
| Respiratory Rate (/min) | 22.12 ± 3.15 | 22.94 ± 3.57 | 1.263 | 0.209 |
| Oxygen Saturation (%) | 95.62 ± 2.14 | 94.48 ± 2.56 | 2.531 | 0.013 |
| PSI (Scores) | 63.45 ± 15.32 | 69.97 ± 15.47 | 2.206 | 0.030 |

Note: PSI: Pneumonia Severity Index.

*Baseline vital signs and PSI scores*

As shown in **Table 2**, oxygenation levels were significantly lower in the deteriorated group than in the improved group (P = 0.013). In addition, PSI scores were significantly higher in the deteriorated group compared to the improved group (P = 0.030). No significant differences were observed between the two groups with respect to body temperature (P = 0.188), heart rate (P = 0.352), or respiratory rate (P = 0.209).

*Baseline inflammatory markers*

Patients in the deteriorated group exhibited significantly higher levels of several inflammatory biomarkers at baseline, including WBC counts ($P = 0.038$), CRP ($P = 0.010$), HMGB1 ($P < 0.001$), IL-6 ($P < 0.001$), and TLR4 ($P < 0.001$), as shown in **Figure 1**.

*Clinical symptoms*

No significant differences were observed between the two groups in the incidence of fever

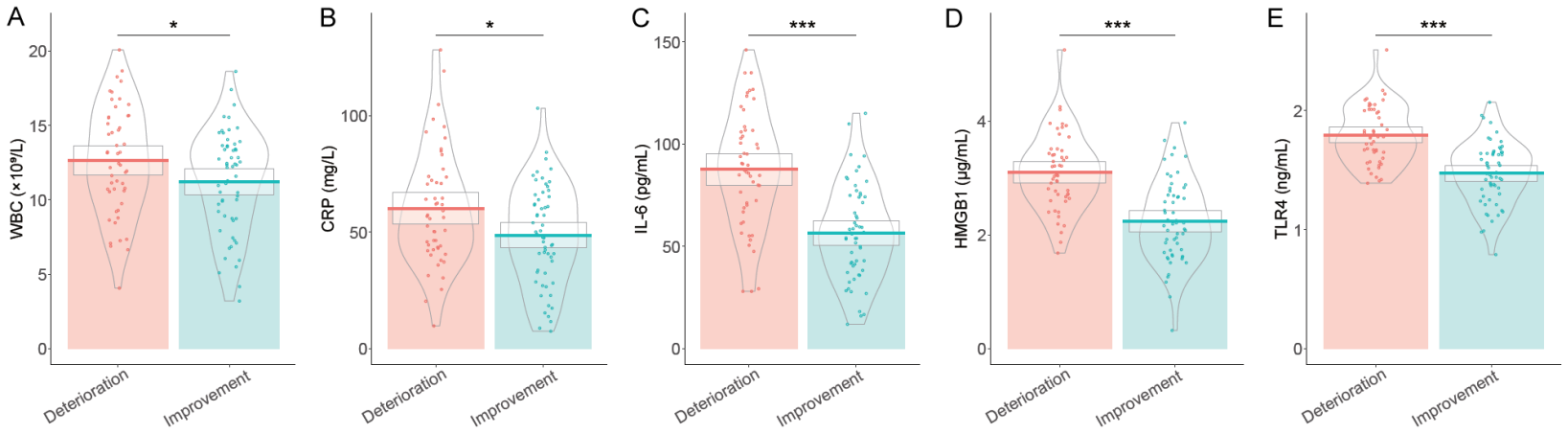


**Figure 1.** Comparison of baseline levels of inflammatory markers between the two groups. A. WBC; B. CRP; C. IL-6; D. HMGB1; E. TLR4. Note: WBC: White Blood Cells; CRP: C-reactive Protein; IL-6: Interleukin-6; HMGB1: High-mobility Group Box 1; TLR4: Toll-like Receptor 4. *$P < 0.05$; ***$P < 0.001$. WBC: White Blood Cells; CRP: C-reactive Protein; IL-6: Interleukin-6; HMGB1: High-mobility Group Box 1; TLR4: Toll-like Receptor 4.

**Table 3.** Comparison of clinical symptoms between the two groups [n (%)]

| Item | Improved Group (n = 58) | Deteriorated Group (n = 51) | $\chi^2$ | P |
|---|---|---|---|---|
| Fever (≥ 38°C) | 32 (55.17) | 28 (54.90) | 0.001 | 0.977 |
| Cough | 50 (86.21) | 45 (88.24) | 0.100 | 0.752 |
| Sputum Production | 38 (65.52) | 35 (68.63) | 0.119 | 0.730 |
| Dyspnea | 28 (48.28) | 25 (49.02) | 0.006 | 0.938 |
| Chest Pain | 12 (20.69) | 10 (19.61) | 0.020 | 0.888 |
| Fatigue | 34 (58.62) | 32 (62.75) | 0.193 | 0.66 |
| Confusion | 5 (8.62) | 4 (7.84) | 0.000 | 1.000 |

**Table 4.** Correlations analysis between various variables and poor prognosis

| Variable | rho | P |
|---|---|---|
| Oxygen Saturation (%) | -0.247 | 0.010 |
| PSI (Scores) | 0.199 | 0.038 |
| WBC (× $10^9$/L) | 0.179 | 0.063 |
| CRP (mg/L) | 0.205 | 0.032 |
| IL-6 (pg/mL) | 0.523 | *P* < 0.001 |
| HMGB1 (μg/mL) | 0.536 | *P* < 0.001 |
| TLR4 (ng/mL) | 0.553 | *P* < 0.001 |

Note: PSI: Pneumonia Severity Index; WBC: White Blood Cells; CRP: C-reactive Protein; IL-6: Interleukin-6; HMGB1: High-mobility Group Box 1; TLR4: Toll-like Receptor 4.

(≥ 38°C; P = 0.977), cough (P = 0.752), expectoration (P = 0.730), dyspnea (P = 0.938), chest pain (P = 0.888), fatigue (P = 0.660), or altered consciousness (P = 1.000) (**Table 3**).

*Correlation analyses*

Lower oxygen saturation (%) was negatively associated with poor prognosis (rho = -0.247; *P* = 0.010). PSI score was positively associated with poor prognosis (rho = 0.199; *P* = 0.038). Among inflammatory markers, CRP was positively associated with poor prognosis (rho = 0.205; *P* = 0.032). Although WBC counts showed a positive tendency, the association did not reach significance (rho = 0.179; *P* = 0.063). In contrast, IL-6 (rho = 0.523; P < 0.001), HMGB1 (rho = 0.536; P < 0.001) and TLR4 (rho = 0.536; P < 0.001) showed strong positive correlations with adverse outcome. Details are shown in **Table 4**.

*Univariate logistic regression analysis*

Univariate logistic regression analysis identified several inflammatory markers significantly associated with prognosis in CAP patients (**Table 5**). WBC count showed a modest association with an increased risk of deterioration (OR = 1.123, *P* = 0.042), while CRP (OR = 1.023, *P* = 0.013) and IL-6 (OR = 1.047, *P* < 0.001) showed positive associations with adverse outcomes. In addition, HMGB1 (OR = 6.164, *P* < 0.001) and TLR4 (OR = 217.654, *P* < 0.001) exhibited strong association with poor prognosis.

*Multivariate logistic regression analysis*

Multivariate logistic regression analysis demonstrated that IL-6, HMGB1, and TLR4 were independent predictors of adverse outcomes in patients with CAP (**Table 6**). IL-6 remained significantly associated with poor prognosis (OR = 1.052, *P* < 0.001). HMGB1 was independently associated with deterioration (OR = 6.769, *P* < 0.001), and TLR4 also showed a significant association with adverse outcome (OR = 66.929, *P* = 0.002). In contrast, WBC count (OR = 1.171, *P* = 0.091) and CRP (OR = 1.008, *P* = 0.626) were not independently associated with prognosis in the multivariate model.

*Predictive performance of inflammatory biomarkers*

ROC analyses were performed for each biomarker (**Figure 2**). The area under the curve (AUC) values were 0.603 for leukocytes, 0.619 for CRP, 0.803 for IL-6, 0.810 for HMGB1, and 0.820 for TLR4. These results suggest that IL-6, HMGB1, and TLR4 have superior discriminative performance compared to WBC and CRP. The combined predictive model incorporating IL-6, HMGB1, and TLR4 showed excellent discriminatory ability, with an AUC of 0.940 (**Figure 3**), markedly higher than the AUCs of IL-6, HMGB1, or TLR4 alone.

**Table 5.** Univariate logistic regression analysis of inflammatory markers for the prognosis in CAP patients

| Risk Factors | Coefficient | SE | Wald | OR (95% CI) | P |
|---|---|---|---|---|---|
| WBC (× $10^9$/L) | 0.116 | 0.057 | 2.037 | 1.123 (1.007-1.262) | 0.042 |
| CRP (mg/L) | 0.022 | 0.009 | 2.480 | 1.023 (1.005-1.042) | 0.013 |
| IL-6 (pg/mL) | 0.046 | 0.010 | 4.767 | 1.047 (1.029-1.069) | < 0.001 |
| HMGB1 (μg/mL) | 1.819 | 0.378 | 4.806 | 6.164 (3.111-13.856) | < 0.001 |
| TLR4 (ng/mL) | 5.383 | 1.122 | 4.796 | 217.654 (29.359-2483.905) | < 0.001 |

Note: CAP: Community-acquired pneumonia; WBC: White Blood Cells; CRP: C-reactive Protein; IL-6: Interleukin-6; HMGB1: High-mobility Group Box 1; TLR4: Toll-like Receptor 4.

**Table 6.** Multivariate logistic regression analysis of inflammatory markers for the prognosis in CAP patients

| Risk Factors | Coefficient | SE | Wald | OR (95% CI) | P |
|---|---|---|---|---|---|
| WBC (× $10^9$/L) | 0.158 | 0.094 | 1.688 | 1.171 (0.975-1.407) | 0.091 |
| CRP (mg/L) | 0.008 | 0.017 | 0.488 | 1.008 (0.975-1.042) | 0.626 |
| IL-6 (pg/mL) | 0.051 | 0.014 | 3.689 | 1.052 (1.024-1.081) | < 0.001 |
| HMGB1 (μg/mL) | 1.912 | 0.565 | 3.385 | 6.769 (2.237-20.480) | < 0.001 |
| TLR4 (ng/mL) | 4.204 | 1.343 | 3.129 | 66.929 (4.809-931.562) | 0.002 |

Note: CAP: Community-acquired pneumonia; PSI: Pneumonia Severity Index; WBC: White Blood Cells; CRP: C-reactive Protein; IL-6: Interleukin-6; HMGB1: High-mobility Group Box 1; TLR4: Toll-like Receptor 4.

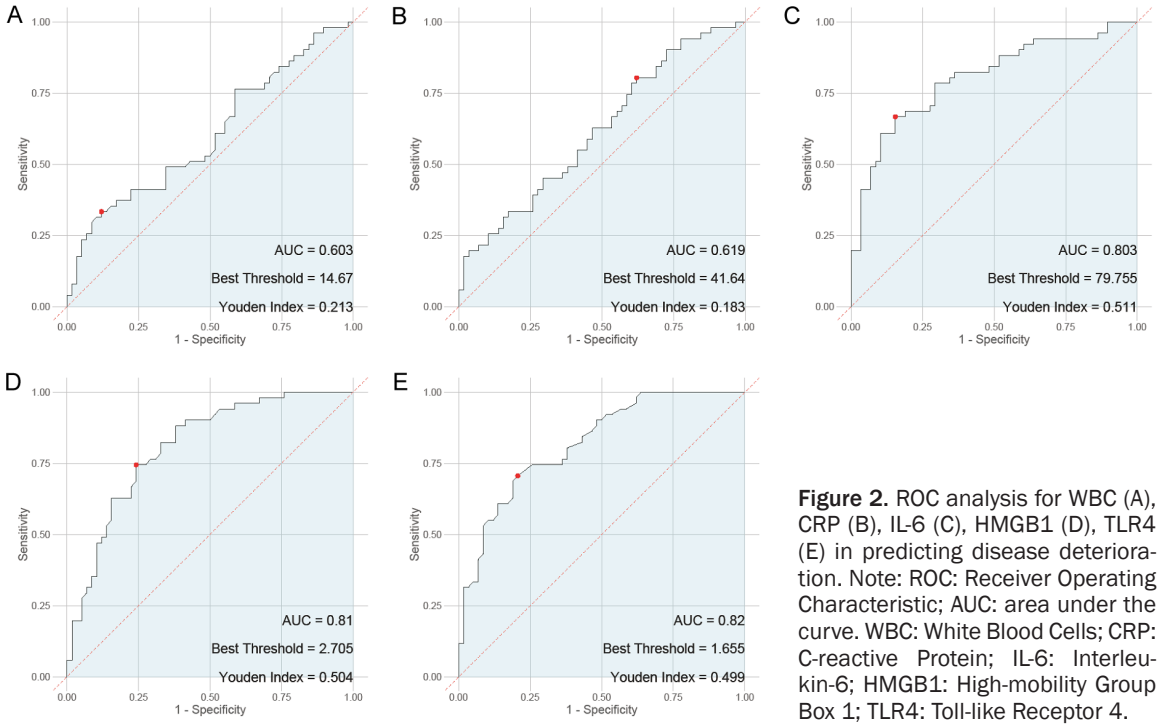


**Figure 2.** ROC analysis for WBC (A), CRP (B), IL-6 (C), HMGB1 (D), TLR4 (E) in predicting disease deterioration. Note: ROC: Receiver Operating Characteristic; AUC: area under the curve. WBC: White Blood Cells; CRP: C-reactive Protein; IL-6: Interleukin-6; HMGB1: High-mobility Group Box 1; TLR4: Toll-like Receptor 4.

DCA was performed to evaluate the clinical use of WBC, CRP, IL-6, HMGB1, and TLR4 for predicting disease deterioration. As presented in **Figure 4**, IL-6, HMGB1, and TLR4 demonstrated higher net benefit across a range of threshold probabilities compared to WBC and CRP.

## Discussion

We investigated whether inflammatory biomarkers in BALF could predict the prognosis of CAP patients. Our results demonstrated that IL-6 was an independent predictor of poor progno-

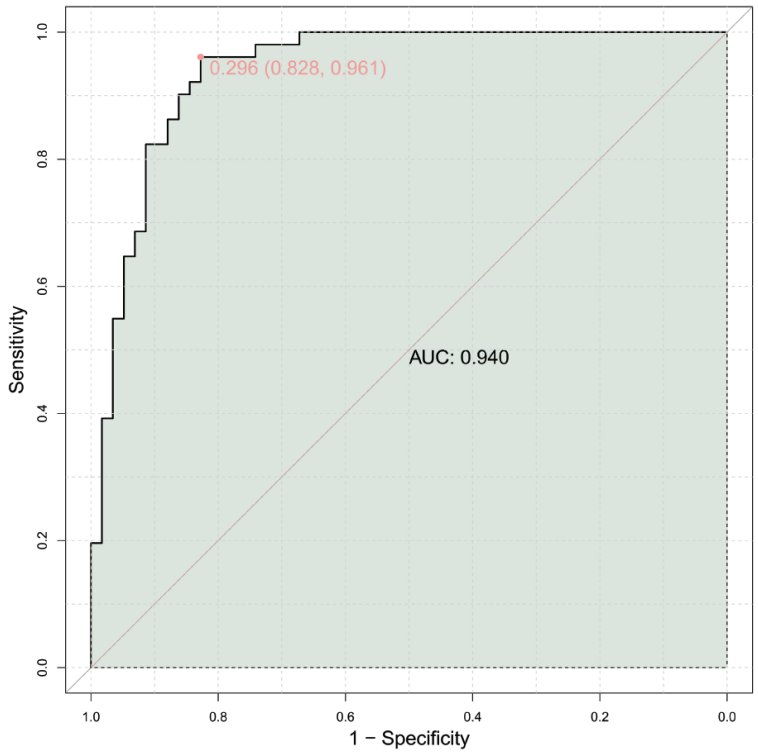


**Figure 3.** ROC analysis for joint assessment of IL-6, HMGB1, and TLR4 in predicting disease deterioration. IL-6: Interleukin-6; HMGB1: High-mobility Group Box 1; TLR4: Toll-like Receptor 4.

sis. This finding is consistent with the established role of IL-6 as a key regulator of systemic inflammation and immune response [21, 22]. IL-6 is a pivotal cytokine that orchestrates the acute-phase response and links between innate and adaptive immunity. In CAP, IL-6 is produced locally by alveolar macrophages and other pulmonary resident cells upon pathogen-associated molecular patterns, driving the hepatic production of CRP and fibrinogen. More critically, IL-6 promotes neutrophil recruitment and activation, influences T-cell differentiation, and modulates regulatory T-cell function. Sustained IL-6 signaling creates a positive feedback loop that amplifies local and systemic inflammation, leading to tissue injury, endothelial dysfunction, and impaired gas exchange. These mechanisms provide a biologic explanation for the association between elevated IL-6 levels in BALF and adverse clinical outcome in CAP [23-25].

HMGB1 also emerged as a robust prognostic marker in this study. HMGB1 functions not only as a nuclear DNA-binding protein but also as a damage-associated molecular pattern (DAMP) when released into the extracellular space [26, 27]. HMGB1 can be passively released from necrotic cells or actively secreted by immune cells in response to inflammatory stimuli. Once released into the extracellular space, HMGB1 interacts with pattern recognition receptors, including TLRs and the receptor for advanced glycation end products (RAGE), thereby initiating and sustaining inflammatory signaling cascades. In this study, HMGB1 was markedly elevated in the deteriorated group, suggesting its role in perpetuating inflammation. Binding of HMGB1 to TLR4 on macrophages and dendritic cells triggers MyD88-dependent signaling pathways, leading to the activation of NF-κB and the production of pro-inflammatory cytokines such as IL-6, TNF-α, and IL-1β. This HMGB1-TLR4 axis may exacerbate pulmonary inflammation, disrupt the alveolar-capillary barrier, promote pulmonary edema, and impair gas exchange [28, 29].

TLR4 emerged as the most powerful prognostic marker. TLRR4 is a canonical pattern recognition receptor primarily responsible for sensing pathogen-associated molecular patterns (PAMPs), particularly lipopolysaccharide. Elevated TLR4 expression in patients with disease deterioration may reflect a higher bacterial load or an enhanced inflammatory response. While TLR4-mediated signaling is essential for effective pathogen clearance, excessive or sustained activation may exert deleterious effects, characterized by excessive cytokine release and subsequent tissue injury, which is frequently observed in severe CAP. Therefore, the strong predictive value of TLR4 may be attributed to its position at the upstream initiation of the inflammatory cascade [30, 31].

Although systemic inflammatory markers such as CRP and WBC exhibited dynamic changes during disease progression, multivariate regression analysis did not identify them as in-

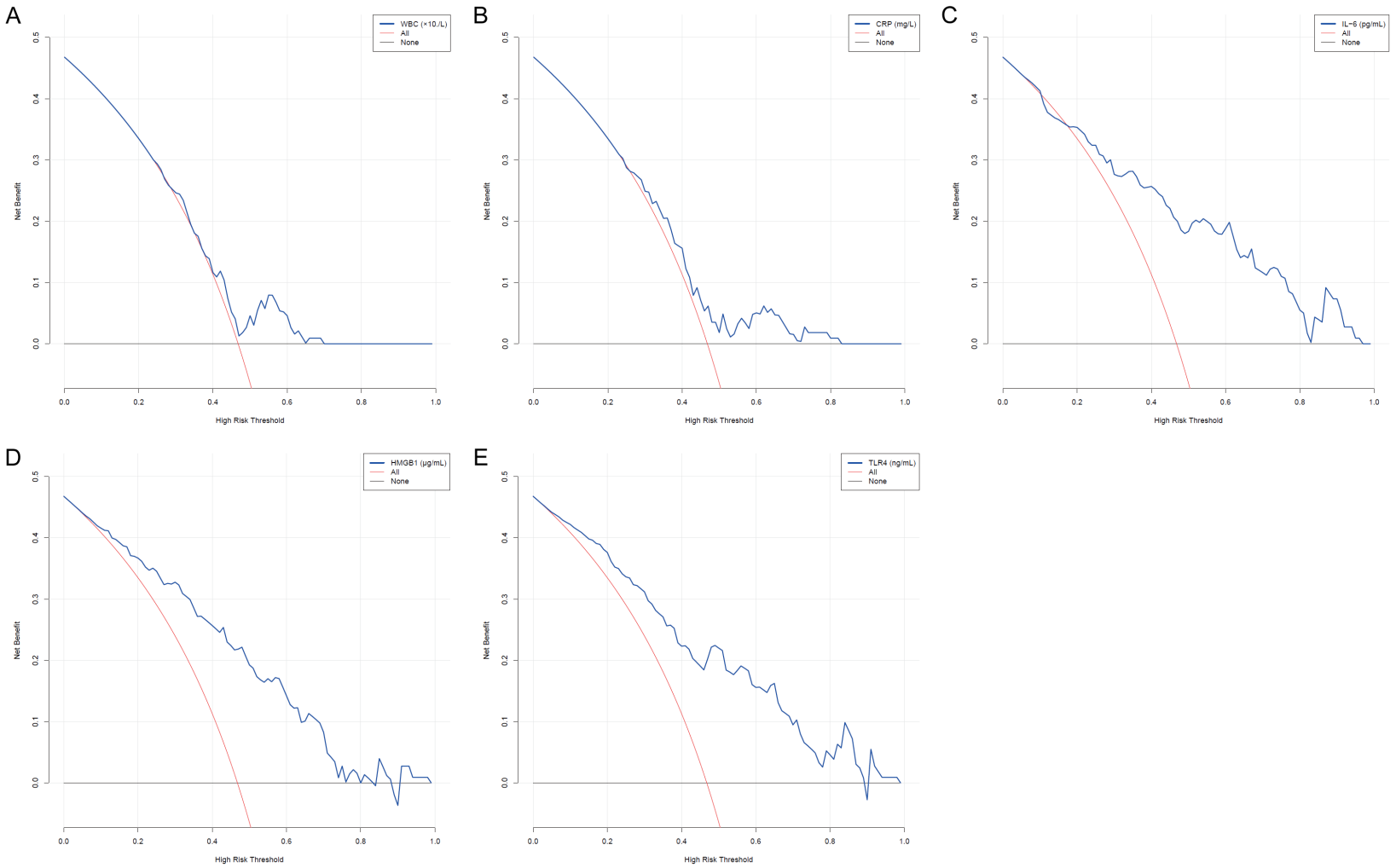


**Figure 4.** DCA for WBC (A), CRP (B), IL-6 (C), HMGB1 (D), TLR4 (E) and disease deterioration. Note: DCA: Decision Curve Analysis. WBC: White Blood Cells; CRP: C-reactive Protein; IL-6: Interleukin-6; HMGB1: High-mobility Group Box 1; TLR4: Toll-like Receptor 4.

dependent predictors of prognosis. A possible reason is that CRP and WBC are nonspecific indicators of systemic inflammation and do not specifically reflect activation of key inflammatory pathways in CAP pathogenesis. Their modest association with clinical outcomes suggests that they lack sufficient discriminatory power compared to pathway-specific biomarkers measured in BALF. This discrepancy further highlights the importance of local pulmonary immune responses over systemic inflammatory markers for determining disease severity and prognosis in CAP [32, 33].

Integrated assessment of these biomarkers offers a multidimensional perspective on CAP pathogenesis, encompassing both protective host defense and maladaptive inflammatory states. While inflammation is essential for pathogen clearance, persistent or excessive activation may lead to tissue injury and unfavorable clinical outcome. Personalized interventions targeting key inflammatory pathways present a promising approach to mitigate such excessive inflammation. Specific IL-6 inhibitors or TLR4 signaling antagonists represent potential strategies to modulate hyperinflammation while preserving protective immune functions. Although these therapeutic approaches require further experimental and clinical validation, they may offer a rationale for tailoring CAP management according to individual inflammatory signatures [34, 35].

ROC analysis and DCA demonstrated that predictive models incorporating IL-6, HMGB1, and TLR4 provide meaningful clinical utility. These biomarkers enabled earlier and more accurate prediction of disease deterioration in patients with CAP, which may facilitate risk stratification and individualized decision-making [36].

Despite these strengths, several limitations of the present study should be acknowledged. First, the retrospective design may have introduced selection bias and precludes definitive causal inference. Although a multivariate logistic regression model adjusted for several key baseline characteristics (e.g., age, BMI, comorbidities), other potential confounders, such as pathogen-specific etiology and detailed prior medication history, were not systematically assessed or controlled. The absence of pathogen-specific stratification may have influenced the observed inflammatory biomarker profiles, given the heterogeneous etiologies of CAP. Second, while comprehensive clinical symptom data were collected, the correlation between symptom severity and corresponding biomarker levels were not analyzed. Such analyses could provide further insight into phenotype-biomarker associations and enhance understanding of disease heterogeneity. Third, the relatively limited sample size may restrict the generalizability of the findings to broader populations. Future studies should adopt a prospective design with larger and more diverse cohorts, incorporate pathogen identification, and apply advanced statistical approaches - such as propensity score matching or stratification based on disease etiology and comorbidity burden - to further refine prognostic models and minimize residual confounders. Longitudinal evaluation of dynamic changes in BALF biomarkers during disease progression and recovery may also offer additional prognostic and mechanistic insight.

## Conclusion

IL-6, HMGB1 and TLR4 in BALF are valuable prognostic biomarkers for patients with CAP. Elevated levels of these biomarkers are strongly associated with disease severity and adverse clinical outcome, suggesting their involvement in inflammatory signaling and immune dysregulation during CAP progression. The combined assessment of IL-6, HMGB1, and TLR4 showed superior predictive performance compared to individual markers, highlighting the advantage of a combined approach.

## Disclosure of conflict of interest

None.

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