Original Research

Prediction of outcome in patients with acute respiratory distress syndrome by bronchoalveolar lavage inflammatory mediators

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Abstract

Acute respiratory distress syndrome (ARDS) is characterized by overwhelming lung inflammation. This study explored the inflammatory mediators in bronchoalveolar lavage fluid (BALF) for prognostic relevance in patients with infection-induced ARDS. Thirty-nine patients with infection-induced ARDS (28 pneumonia and 11 extrapulmonary sepsis) and two patients with cardiogenic lung edema as the control were included. The expression profiles of inflammatory mediators in BALF were compared between ARDS and cardiogenic lung edema. A group of inflammatory mediators that showed higher expression in ARDS was analyzed for their relationships with clinical features and outcome. We found that 17 patients who died had higher levels of interleukin (IL)-6 ($P = 0.012$), IL-8 ($P = 0.001$) and monocyte chemoattractant protein-1 ($P = 0.036$) in BALF compared with those who survived. Furthermore, there was an inverse relationship between the BALF levels of IL-6 ($P = 0.026$), IL-8 ($P = 0.008$) and macrophage inflammatory protein (MIP)-1α ($P = 0.048$) and the changes of lung compliance between days 1 and 4, whereas the BALF levels of IL-8 ($P = 0.033$) and MIP-1α ($P = 0.029$) were positively correlated with the changes of sequential organ failure assessment scores between days 1 and 4. In multivariate logistic regression analysis, only IL-8 ($P = 0.013$) and lung injury score (LIS) ($P = 0.017$) independently predicted the mortality, and IL-8 ($P = 0.002$) was most likely predictive of mortality in analysis of area under the receiver operating characteristic curve. In conclusion, we show the expression profiles of inflammatory mediators in BALF of infection-induced ARDS. Among the mediators, IL-8 is the most significant predictor for mortality, and several mediators are correlated with clinical severity. However, potential selection bias due to limited control subjects and lack of serum inflammatory mediator data suggest a necessity of further studies to confirm our findings.

Keywords: acute respiratory distress syndrome, bronchoalveolar lavage fluid, inflammatory mediators, lung injury score, Δcompliance, ΔSOFA

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Introduction

Acute respiratory distress syndrome (ARDS) is characterized by overwhelming lung injury and inflammation and increased microvascular permeability that causes diffuse lung edema and mechanical dysfunction leading to respiratory failure.[1–4] ARDS is a major leading cause of mortality and morbidity in adult patients admitted to intensive care units (ICU). The mortality remains high (approximately 40%), although recent data have shown a reduction in mortality rates since lung protective ventilatory strategies were implemented.[5–7] The main cause of death in patients with ARDS is multiple organ failure, which might be caused by a systemic inflammation in response to lung injury.[8,9] During ARDS, the alveolar epithelial–endothelial barrier is disrupted, and inflammatory cytokines produced in the lung are released into the systemic circulation, which is proposed to be related to the development of multiple organ dysfunctions.[10] Therefore, determination of the inflammatory mediators in bronchoalveolar lavage fluid (BALF) from ARDS patients can be of prognostic relevance.[11,12] Several inflammatory mediators have been recognized to be important in the pathophysiology of sepsis, a condition frequently leading to ARDS.[13] In the lung, inflammatory mediators can be generated either by local resident cells such as alveolar macrophages, epithelial cells, endothelial cells and fibroblasts or by immigrant cells such as

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neutrophils, lymphocytes and platelets in response to local or systemic inflammation.[14] Inflammatory mediators involved in the early phase of ARDS include cytokines and chemokines, such as tumor necrosis factor-α, interleukin (IL)-1, IL-6, IL-8 and monocyte chemoattractant protein (MCP)-1.[15,16] The predictive value of these inflammatory mediators for the outcome of patients with ARDS has been reported, but the results are inconclusive.[10–12,17] In addition, there were only limited inflammatory mediators included for evaluation in each study, and the profiles of inflammatory mediators in BALF from ARDS patients remain unclear. Because ARDS frequently complicates the clinical course of severe sepsis and pneumonia, various inflammatory mediators in BALF are likely related to the outcome of infection-induced ARDS. We, therefore, investigated a panel of inflammatory mediators, of which the expression levels were higher in ARDS than in cardiogenic pulmonary edema, as possible prognostic factors for the severity and outcome of patients with ARDS.

Materials and methods

Patients

Patient samples were collected between July 2005 and July 2006 at the medical ICU of the tertiary referral center of southern Taiwan. We prospectively studied 39 consecutive patients receiving mechanical ventilation who were admitted to ICU and diagnosed with ARDS, defined as the presence of the following standard criteria: (1) acute hypoxemic respiratory failure; (2) diffuse bilateral alveolar infiltrates on the chest radiograph; (3) refractory hypoxemia with arterial partial pressure of oxygen/fraction of inspired oxygen (PaO_2/FiO_2) < 200, regardless of positive end-expiratory pressure level; (4) pulmonary artery wedge pressure <18 mm H_2O or no clinical evidence for left atrial hypertension; and (5) recognized appropriate clinical setting or risk factor for the development of ARDS.[1] All of these patients had either extrapulmonary sepsis ($n = 11$) or pneumonia ($n = 28$). Sepsis was defined according to the published consensus guidelines,[18] and pneumonia was diagnosed if the following criteria were met: (1) symptoms and signs of acute lower respiratory tract infection; (2) a new or progressive pulmonary infiltrate on chest radiography; and (3) identification of microbes in the lower respiratory tract. Patients were excluded if they were <16 years of age, had refractory respiratory failure (PaO_2 <60 mmHg with $FiO_2 = 1.0$) or had unstable hemodynamic status and lethal arrhythmia even under the use of a high-dose vasopressor and antiarrhythmia drug. All patients received treatment for ARDS using protocols that followed lung protective strategy [7] and the Surviving Sepsis Campaign guidelines.[19] Empirical board-spectrum antibiotics were started as early as possible in all patients. Once causative pathogens were defined, the antimicrobial regimen was adjusted based on the antibiotic susceptibilities and infectious specialists' suggestions. Antimicrobial regimen and cultures were considered to be re-assessed and re-examined in patients who had a poor clinical response or deteriorated condition. We also included patients with cardiogenic lung edema ($n = 2$), requiring mechanical ventilation and absence of lung or systemic inflammation, as the control subjects. The protocols and procedures were approved by the institutional review board of the National Cheng Kung University Hospital.

The baseline characteristics such as age and gender, laboratory data including PaO_2/FiO_2 ratio, neutrophil count and total protein level in BALF and parameters of lung mechanics such as compliance were recorded on the first day of ARDS onset. Hemodynamic data, ventilator parameters and laboratory data were collected and the worst daily values for all variables of interest were recorded to calculate the sequential organ failure assessment (SOFA) score,[20] acute physiology and chronic health evaluation II (APACHE II) score[21] and lung injury score (LIS) as defined by Murray and colleagues[22] in the first 24 h of ARDS onset. Lung compliance and SOFA scores on day 4 were also recoded for calculating the changes between days 1 and 4 (Δcompliance and ΔSOFA).

Fiberoptic bronchoscopy sampling

All patients included in the study underwent fiberoptic bronchoscopy (Olympus LF2 or P40; Olympus Optic, Tokyo, Japan) within 24 h once the diagnosis of ARDS was established. All the patients were mechanically ventilated with FiO_2 100%, sedated with midazolam and paralyzed with atracurium. No topical anesthetics were used before BAL. The fiberoptic bronchoscope was introduced without bronchial suctioning, except after BAL. Heart rate, blood pressure and arterial oxygen saturation were monitored throughout the procedure. The bronchoscope was wedged into the bronchus of the right middle lobe or lingular division, or the area of pneumonia. Six aliquots (20 mL each) of sterile normal saline were instilled and the fluid was aspirated immediately after each instillation. The first retrieved BALF, reflecting a bronchial sample, was discarded and the remaining BALF was pooled in ice-cold tubes for study. A portion of the BALF from each patient was used for the cellular analysis. The total protein concentrations in the BALF were measured using the Biuret method. Total cell numbers were counted using a hemocytometer, and the cell differential was measured on cytospin preparations using Liu's staining. A minimum of 300 cells were examined. The BALF was centrifuged at 200*g* for 10 min at 4°C to obtain the supernatant. Aliquots of the cell-free supernatant were stored at -80°C until assay.

Human protein cytokine array

A human protein cytokine array kit was purchased from RayBiotech (Norcross, GA, USA). Briefly, the membrane was blocked with a blocking buffer, and then 1 mL of BAL supernatant was added and incubated at room temperature for two hours. The membrane was washed and 1 mL of primary biotin-conjugated antibody was added and incubated at room temperature for two hours. The membrane was incubated with 2 mL of horseradish peroxidase-conjugated streptavidin at room temperature for 30 min. The membrane was developed using enhanced

chemiluminescence solution, exposed to film, followed by autoradiography.

BALF cytokine/chemokine enzyme-linked immunosorbent assay

The concentrations of IL-1β, IL-6, IL-6-soluble receptor (IL-6sR), IL-8, MCP-1, MCP-2, macrophage inflammatory protein (MIP)-1α, MIP-1δ, interferon-inducible protein-10 (IP-10) and tissue inhibitors of metalloproteinases-2 (TIMP-2) in BALF were measured in duplicate using solid phase sandwich enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions.

Statistical analysis

Statistical analyses were performed using a statistical software package (SPSS for Windows, version 13.0; SPSS Inc, Chicago, IL, USA). Descriptive frequencies were expressed using median (range). Differences between the means of continuous variables were compared using the Mann-Whitney U test, and those of categorical variables were compared with the χ^2 test. Levels of significance were expressed as P values. Stepwise multivariate logistic regression analysis was performed to analyze the independent risk factors for hospital mortality. Initially, the variables (i.e. age, gender, etiology, baseline condition, laboratory data, lung compliance, SOFA score, APACHE II score and LIS score) were analyzed using univariate analysis. The variables that showed significant or nearly significant differences (i.e. $P < 0.1$) on univariate analysis were then entered in a multivariate logistic regression model to derive the independent prognostic factors. Spearman's correlation of rank coefficient was used to analyze correlations between the inflammatory mediators and the various parameters measured. The area under the receiver operating characteristic (ROC) curve was used to evaluate the ability to discriminate between patients who survived and those who died.[23] A two-sided P value <0.05 was considered to be statistically significant.

Results

Thirty-nine patients with ARDS receiving mechanical ventilation during the study period were enrolled in the study. The causes of ARDS were primary pneumonia ($n = 28$) and sepsis of extrapulmonary origin ($n = 11$). The in-hospital mortality was 43.6%(17/39). There was no significant difference between survivors and non-survivors in age, gender, proportion of patients with pneumonia to extrapulmonary sepsis, APACHE II and SOFA scores and lung compliance at the time of ARDS onset (Table 1). The patients who died in the hospital had significantly higher total protein levels in BALF and LIS scores, lower PaO_2/FiO_2 at the time of ARDS onset and lung compliance on day 4 of ARDS onset and higher percentages of positive blood cultures compared with those who survived (Table 1). The micro-organisms found in blood cultures are shown in Table 2.

The profiles of inflammatory mediators in BALF from patients with ARDS were compared with those from patients with cardiogenic lung edema (Figure 1a), and the expression intensity on the membrane of protein cytokine array was shown (Figure 1b). The expression levels of cytokine/cytokine receptor (IL-1β, IL-6, IL-6sR), chemokine (IL-8, IP-10, MCP-1, MCP-2, MIP-1α, MIP-1δ) and tissue inhibitor (TIMP-2) were higher in patients with ARDS than in patients with cardiogenic lung edema. We further measured these molecules using ELISA, in order to analyze factors that might have prognostic value for the outcome of patients with ARDS. We found that 17 patients who died had higher levels of IL-6 ($P = 0.012$), IL-8 ($P = 0.001$) and MCP-1 ($P = 0.036$) in BALF compared with those who survived (Table 3). The levels of IL-1β and

Table 1 Comparisons between survivors and non-survivors of ARDS*

Variables	Survivors (n = 22)	Non-survivors (n = 17)	P value
Age (y)	73 (34–92)	65 (17–79)	0.077
Gender, male (n)	14 (64%)	13 (76%)	0.609
Pneumonia/extrapulmonary sepsis (n)	13/9	15/2	0.073
BAL			
Recovery amount of normal saline	50 (40–80)	50 (30–70)	0.878
Neutrophil count ($\times 10^3$ cells/mL)†	167 (6–1222)	356 (6–12702)	0.206
Total protein (mg/dL)	45 (10–221)	117 (13–299)	0.003
APACHE II	28 (14–43)	27 (17–41)	0.900
SOFA			
Day 1	10 (7–15)	11 (5–20)	0.440
Day 4	9 (4–14)	12 (4–21)	0.067
PaO_2/FiO_2	126 (47–432)	80 (43–135)	0.001
LIS	2.6 (1.3–3.5)	2.8 (2.3–3.8)	0.031
Compliance (mL/cm H_2O)			
Day 1	22 (17–53)	22 (9–35)	0.220
Day 4	29 (16–58)	18 (9–33)	0.001
Micro-organisms in blood (n)	5 (23%)	10 (59%)	0.049

ARDS, acute respiratory distress syndrome; APACHE, acute physiology and chronic health evaluation; SOFA, sequential organ failure assessment; LIS, lung injury score

*Data are presented as number (%) or median (range)

†Data of neutrophil counts from four survivors and three non-survivors were unavailable due to blood cell clotting

Table 2 Micro-organisms from blood cultures in ARDS patients

Micro-organisms	No. of patients
Oxacillin-resistant *Staphylococcus aureus*	2
Oxacillin-sensitive *Staphylococcus aureus*	1
Staphylococcus haemolyticus	1
Vancomycin-resistant *Enterococci*	1
Enterococcus faecalis	2
Enterobacter cloacae	1
Acinetobacter baumannii	1
Burkholderia pseudomallei	1
Klebsiella pneumoniae	3
Chryseobacterium meningosepticum	1
Bacteroides distasonis	1
Candida albicans	1
Candida glabrata	1

ARDS, acute respiratory distress syndrome

MCP-2 in most patients were very low and their concentrations were outside the reliable detection range (Table 3).

IL-8 is a chemoattractant for neutrophils. Our results confirmed that the BALF IL-8 levels were positively correlated with the neutrophil counts in BALF ($\gamma = 0.699$, $P < 0.0001$) (Figure 2). The BALF IL-6 ($\gamma = 0.582$, $P < 0.0001$), IL-8 ($\gamma = 0.408$, $P = 0.010$), MCP-1 ($\gamma = 0.622$, $P < 0.0001$), MIP-1α ($\gamma = 0.328$, $P = 0.045$) and IL-6sR ($\gamma = 0.579$, $P = 0.0001$) levels were significantly correlated with the total protein concentrations in BALF from ARDS patients (Figure 3). We also analyzed the relationship between inflammatory mediators and clinical parameters such as the changes of lung compliance (Δcompliance) and SOFA score (ΔSOFA) between days 1 and 4 of ARDS diagnosis. We found that the BALF IL-6 ($\gamma = 0.366$, $P = 0.026$), IL-8 ($\gamma = 0.432$, $P = 0.008$) and MIP-1α ($\gamma = 0.332$, $P = 0.048$) levels were negatively correlated with Δcompliance (Figure 4). In contrast, the BALF IL-8 ($\gamma = 0.342$, $P = 0.033$) and MIP-1α ($\gamma = 0.354$, $P = 0.029$) levels were positively correlated with ΔSOFA (Figure 4). Although not statistically significant, there was a trend of positive correlation of IL-6 levels with ΔSOFA.

In a multivariate analysis model we found that only BALF IL-8 ($P = 0.013$) and LIS ($P = 0.017$) were independent factors for death. Furthermore, we employed analysis of area under the ROC curve to evaluate the predictive values of IL-6, IL-8, MCP-1 and LIS for surviving as shown in Table 4.

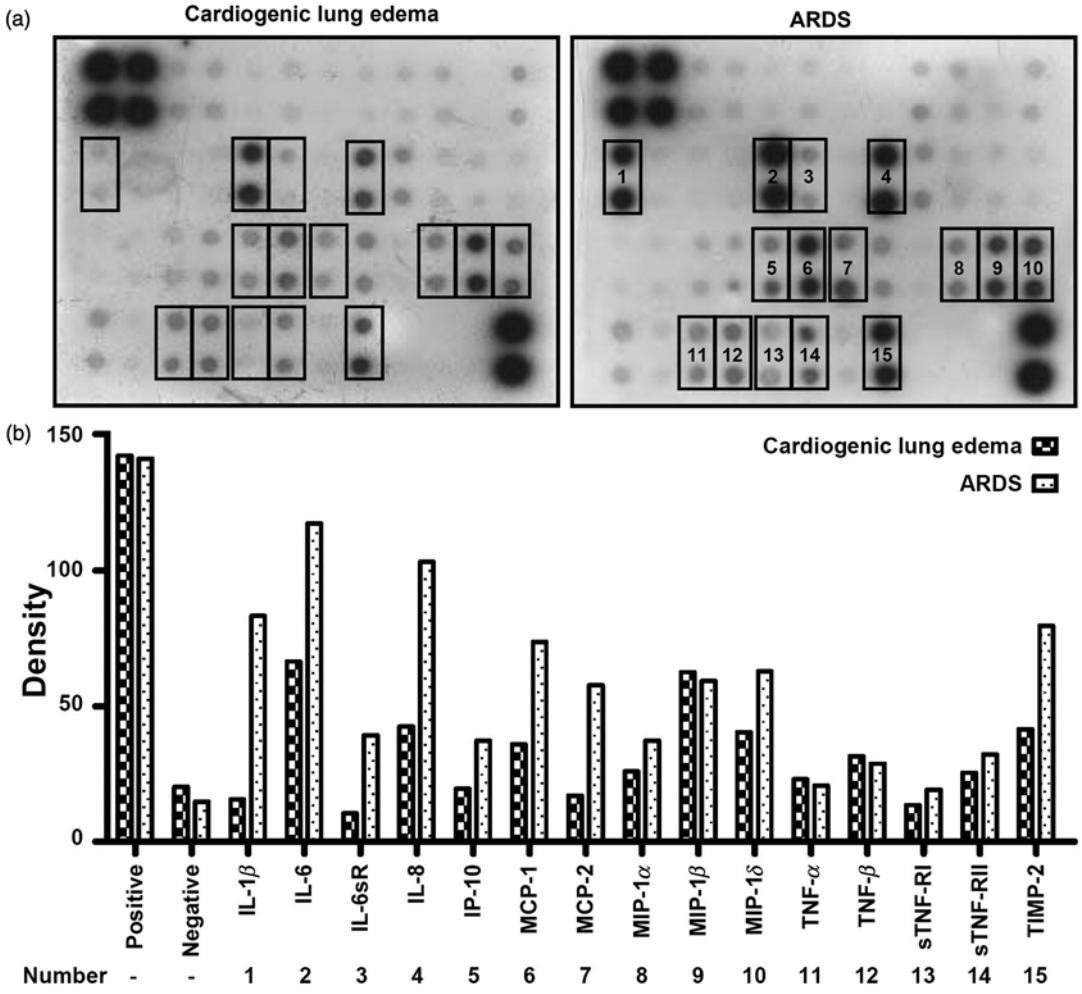

Figure 1 Human cytokine array chips treated with BALF from patients with acute respiratory distress syndrome (ARDS) are compared with those treated with BALF from patients with cardiogenic lung edema. (a) One representative array from two independent experiments. (b) Expression levels of inflammatory mediators shown on membranes were analyzed by densitometry. BALF, bronchoalveolar lavage fluid

Table 3 Comparisons of inflammatory mediators between survivors and non-survivors of ARDS*

Inflammatory mediators (pg/mL)	Survivors (n = 22)	Non-survivors (n = 17)	P value
IL-1β	0 (0–370)	0 (0–398)	0.172
IL-6	35 (0–303)	152 (0–447)	0.012
IL-6sR	132 (11–336)	180 (21–286)	0.504
IL-8	410 (36–1798)	1461 (98–2842)	0.001
MCP-1	191 (16–1530)	705 (26–2226)	0.036
MCP-2	0 (0–389)	0 (0–446)	0.416
MIP-1α	30 (0–185)	51 (0–145)	0.189
MIP-1δ	1950 (71–10760)	1683 (169–3962)	0.685
IP-10	123 (6–1534)	158 (22–1712)	0.467
TIMP-2	681 (45–2317)	714 (246–2294)	0.267

ARDS, acute respiratory distress syndrome; IL, interleukin; IL-6sR, IL-6 soluble receptor; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; IP-10, interferon-inducible protein-10; TIMP-2, tissue inhibitors of metalloproteinases-2
*Data are presented as median (range)

Discussion

The development of ARDS is associated with several clinical disorders including direct pulmonary injury from pneumonia and aspiration as well as indirect pulmonary injury from trauma, sepsis and other disorders such as acute pancreatitis and drug overdose.[1–4] In the present study, we investigated solely patients with ARDS caused by pneumonia or extrapulmonary sepsis in order to focus on the impacts of infection-induced inflammatory mediators in the lung compartment on patient outcome.

We found that the non-survivors had higher BALF total protein levels and LIS scores, lower initial PaO_2/FiO_2 and lung compliance on day 4 after the onset of ARDS, while there were no differences in clinical severity scores (APACHE II and SOFA) between survivors and non-survivors. This result implies that the severity of lung injury is critical with regard to the outcome of ARDS patients, particularly in the similar underlying conditions between survivors and non-survivors, and BALF inflammatory mediators might provide better prediction of outcome than clinical physiological variables. In view of complicated inflammatory mechanisms in the lung compartment during ARDS, it seems difficult to predict outcome based on individual inflammatory molecules. Thus, we examined a group of inflammatory mediators that were remarkably higher in BALF of patients with ARDS, as compared with those of patients with cardiogenic pulmonary edema, for their associations with the outcome of infection-induced ARDS. We found elevated BALF levels of IL-1β, IL-6, IL-6sR, IL-8, IP-10, MCP-1, MCP-2, MIP-1α, MIP-1δ and TIMP-2 in patients with ARDS. This profile of inflammatory mediators clearly separated those patients with inflammatory lung injury from patients with comparable functional impairment due to cardiogenic pulmonary edema.

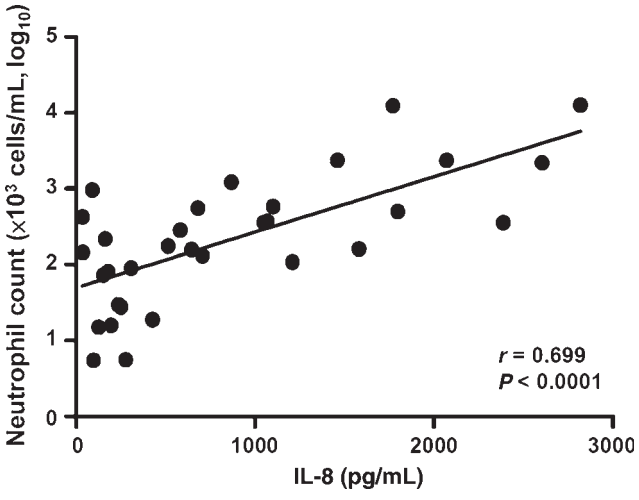

Figure 2 IL-8 levels are positively correlated with neutrophil counts in the BALF from patients with acute respiratory distress syndrome. Data of neutrophil counts from seven patients are unavailable due to blood cell clotting. The IL-8 concentrations in the BALF of the other 32 patients were determined using enzyme-linked immunosorbent assay. IL, interleukin; BALF, bronchoalveolar lavage fluid

IL-8 not only has a role in neutrophil chemotaxis, but also inhibits neutrophil apoptosis.[24,25] Consistent with previous reports,[16,24] we found that IL-8 levels were correlated with the neutrophil counts in BALF from ARDS patients. Regarding outcome prediction, we found that the levels of IL-6, IL-8 and MCP-1 in BALF were higher in non-survivors than in survivors. Significant differences in the BALF IL-6, IL-8 and MCP-1 levels between survivors and non-survivors have been reported in some previous studies,[10,12,17] but not in others.[16,24,26,27] IL-6 can be produced by airway epithelial cells and activates pulmonary macrophages in response to a variety of infectious agents and other inflammatory mediators.[28] IL-8 and MCP-1 are major chemoattractants for neutrophils and monocytes and are expressed by alveolar epithelial cells.[29,30] Both have been reported to take part in the regulation of lung inflammation and cell apoptosis.[16,25,31] In the present study, we found no significant differences in BALF levels of IL-1β, IL-6sR, MCP-2, MIP-1α, MIP-1δ, IP-10 and TIMP-2 between survivors and non-survivors. We have also determined the BALF IL-10 levels, but they were low in both survivors (0–54 pg/mL) and non-survivors (0–57 pg/mL) with a P value of 0.467. However, a previous study showed higher levels of IL-10 in BALF from ARDS patients who died.[17] In contrast, low BALF IL-10 levels were found to be associated with increased mortality in other studies.[32,33] These discrepancies may be explained by differences in the studied population and disease stage among those studies. IP-10 is a chemoattractant for Th1 cells for the activation of cell-mediated immune response and is produced by bronchial epithelial cells in response to infection.[34] IP-10 has been recognized as an independent predictor of outcome during the early stage of severe acute respiratory syndrome.[35–37] We did not find significant differences in IP-10 levels in the BALF between survivors and non-survivors of ARDS patients. In agreement with previous studies,[38–40] concentrations of TIMP-2, the inhibitor of matrix metalloproteinases, were not associated with the outcome of ARDS patients.

In analyzing the relationships with clinical features, the IL-6, IL-8, MCP-1, MIP-1α and IL-6sR levels were positively correlated with the concentrations of total protein in BALF. The correlation of BALF IL-6 and IL-8 levels with total protein concentrations has also been reported previously.[12]

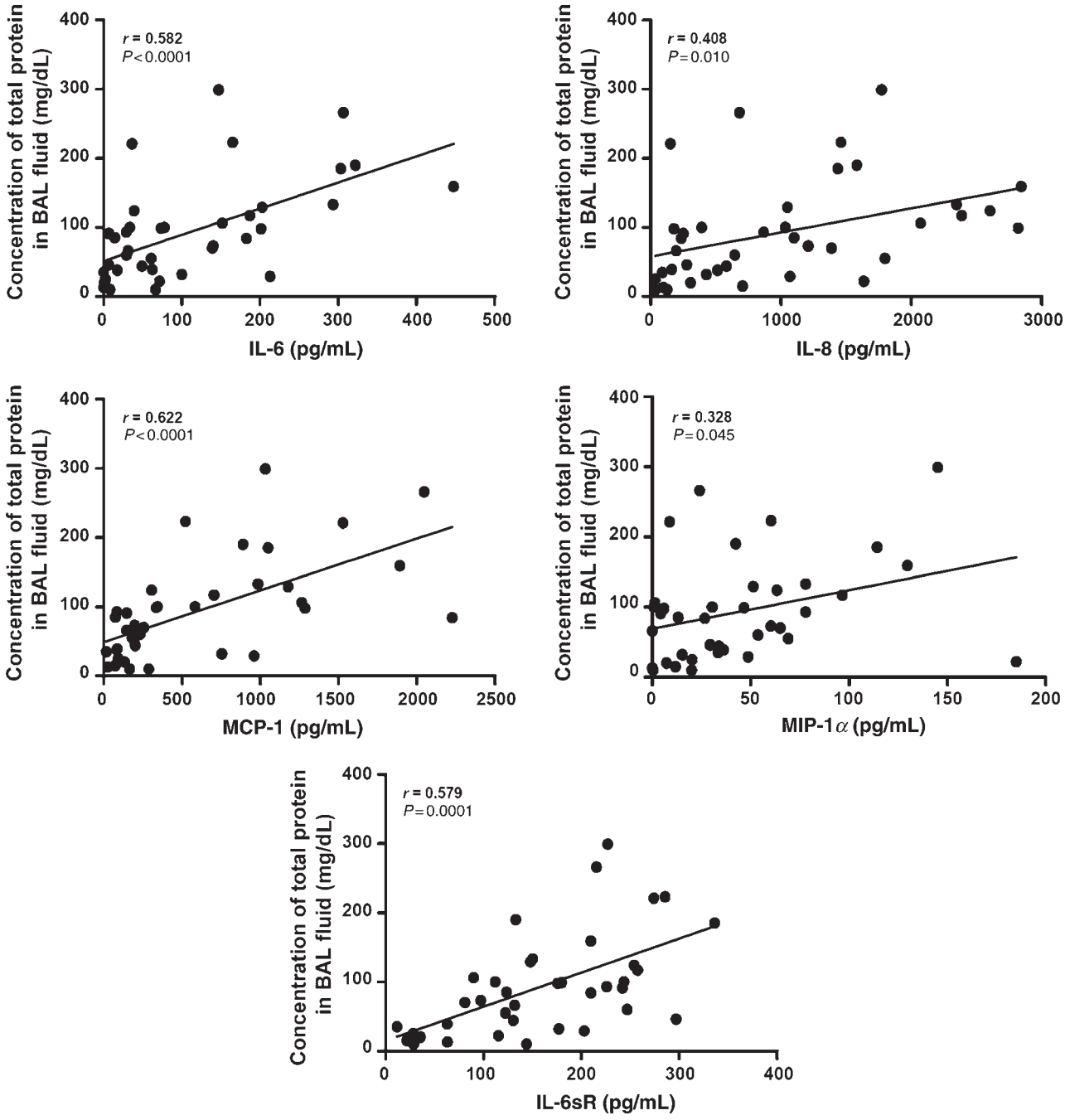

Figure 3 BALF levels of IL-6, IL-8, MCP-1, MIP-1α and IL-6sR are positively correlated with the BALF concentrations of total protein in acute respiratory distress syndrome patients. The IL-6, IL-8, MCP-1, MIP-1α and IL-6sR concentrations in the BALF were determined using enzyme-linked immunosorbent assay. The total protein concentrations in the BALF were measured using the Biuret method ($n = 39$). BALF, bronchoalveolar lavage fluid; IL, interleukin; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein

BALF total proteins reflect the severity of lung inflammation that results in an increase in the permeability of the alveolar–capillary barrier, thus influx of protein from circulation. We further showed that the BALF levels of IL-6, IL-8 and MIP-1α were negatively correlated with the changes of lung compliance between days 1 and 4 of ARDS onset, suggesting that these inflammatory mediators delay the clearance of alveolar fluids and predict deteriorated lung mechanics. The IL-8 and MIP-1α levels in BALF were positively correlated with the changes of SOFA scores between days 1 and 4 of ARDS onset. These mediators, which are closely related to the extension of tissue damage, may be released into systemic circulation to worsen multiple organ dysfunctions. However, no correlation was found between BALF levels of inflammatory mediators and the initial APACHE II, SOFA and LIS scores. This result agrees with a previous report,[12] but not others.[10,17] We suggest that BALF inflammatory molecule

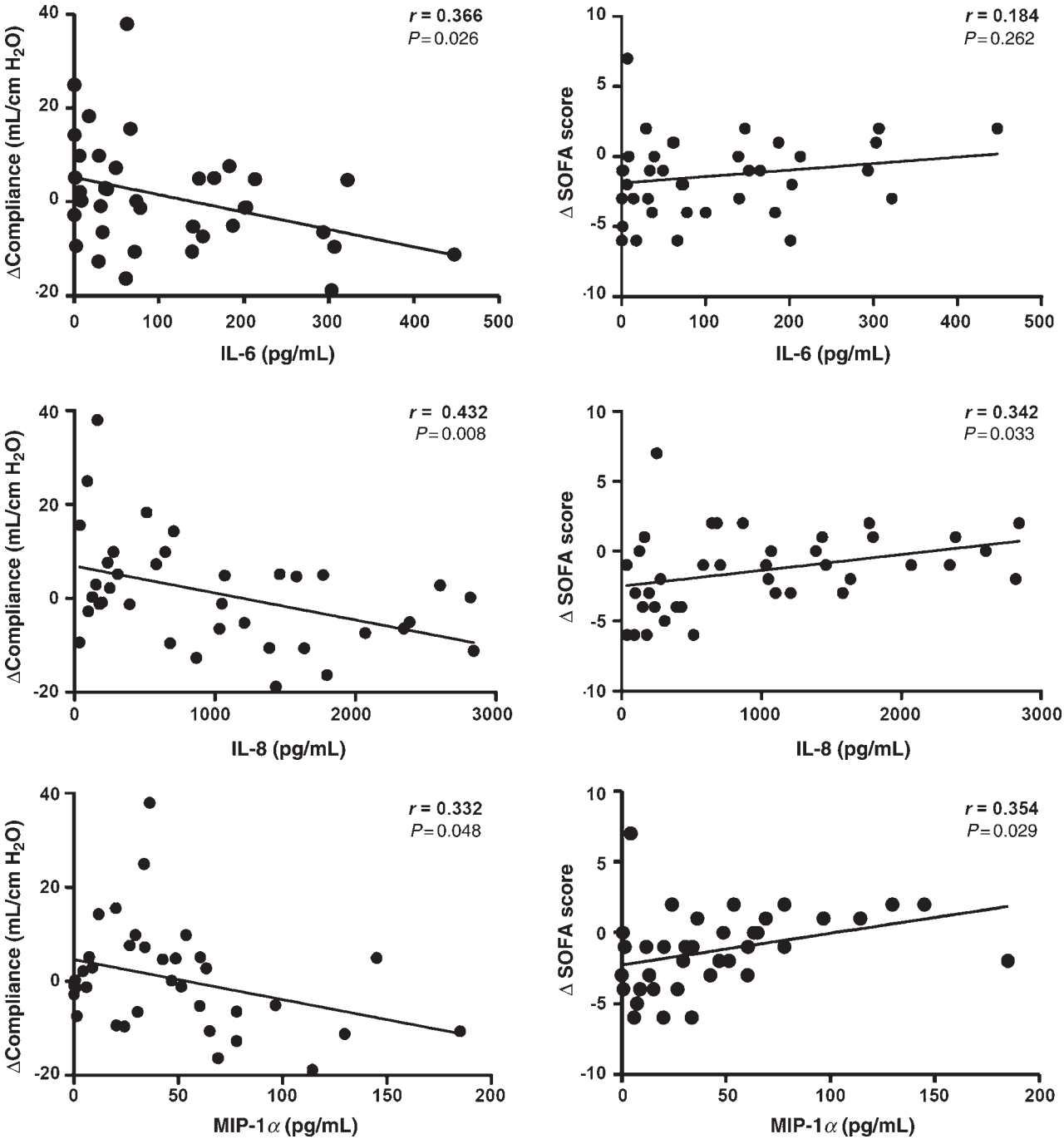

Figure 4 Inverse or positive relationship between BALF levels of IL-6, IL-8 and MIP-1α with Δcompliance and ΔSOFA scores in acute respiratory distress syndrome patients. The IL-6, IL-8 and MIP-1α concentrations in the BALF were determined using enzyme-linked immunosorbent assay. Lung compliance and SOFA scores were recoded on days 1 and 4 to determine the changes between these two days (Δcompliance and ΔSOFA) ($n = 39$). BALF, bronchoalveolar lavage fluid; IL, interleukin; MIP, macrophage inflammatory protein; SOFA, sequential organ failure assessment

Table 4 ROC curve analysis for mortality prediction in ARDS

Variables	Cut-off value	Sensitivity	Specificity	AUC (95% CI)	*P* value
IL-6 (pg/mL)	119	71	86	0.73 (0.56–0.91)	0.013
IL-8 (pg/mL)	1155	65	86	0.79 (0.65–0.95)	0.002
MCP-1 (pg/mL)	244	77	64	0.70 (0.53–0.87)	0.036
LIS	2.38	94	64	0.70 (0.54–0.87)	0.031

ARDS, acute respiratory distress syndrome; ROC, receiver operating characteristic; AUC, area under the ROC curve; CI, confidence interval; IL, interleukin; MCP-1, monocyte chemoattractant protein-1; LIS, lung injury score

levels are related to the changes of clinical physiological variables during a period of time rather than merely the initial value. This reinforces the suggestion that they might be used to predict clinical course in patients with ARDS. Of the BALF inflammatory mediators, only IL-8 along with the LIS was independently associated with mortality in multivariate analysis. The LIS, indicating the severity of lung injury based on the clinical and physiological variables, has been reported to be a significant prognostic factor in one study,[41] but not in another study.[12] However, in the analysis of area under the ROC curve, IL-8 was most likely predictive of mortality as compared with IL-6, MCP-1 and LIS for patients with ARDS, confirming the previous observations.[12,17]

The main strength of this study is that we measured a group of inflammatory mediators in BALF simultaneously in patients with infection-induced ARDS in order to clarify the profiles of inflammatory mediators involved in the pathogenesis of ARDS and the relationship between these molecules and outcome. The major limitations of our study include its small sample size in a single medical center, subsequently preventing us from a subgroup analysis to further examine the similarities in lung inflammatory processes between pneumonia- and extrapulmonary sepsis-induced ARDS,[27] lack of generalizability of these results due to the restriction of our enrollment criteria and some bias in the selection of studied inflammatory mediators based on the repetitive comparison of the expression profiles of inflammatory mediators between ARDS and cardiogenic pulmonary edema. Furthermore, we did not measure the serum levels of the inflammatory mediators. Future studies including more subjects and comparing levels of mediators in serum and BALF in a serial manner would be desirable for further substantiation of our findings.

In conclusion, the present study provides an expression profile of inflammatory mediators involved in the lung compartment of infection-induced ARDS patients and the predictive value of IL-6, IL-8, MCP-1 and LIS for mortality. Both IL-8 and LIS are independent predictors for death. In addition, we show that some inflammatory mediators in BALF are negatively or positively correlated with the changes of clinical physiological variables, that is, Δcompliance and ΔSOFA, respectively.

Statement of author contributions: All authors participated in planning the study and preparing the manuscript; WCL and CWC designed the study and recruited the patients, WCL conducted a significant proportion of the experiments and wrote the manuscript, CLC performed the cytokine array assay and analyzed data, CFL co-designed the study and analyzed data and YSL co-designed the study and reviewed the manuscript.

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