

Association between elevation of plasma biomarkers and monocyte dysfunction and their combination in predicting sepsis: An observational single-centre cohort study

Jiabao Li¹, Ziren Tang¹, Miaorong Xie², Chenchen Hang¹, Yanan Yu¹ and Chunsheng Li¹ 

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Abstract

This study aimed to investigate the possible relationship between the two biomarkers presepsin and procalcitonin (PCT) and monocyte immune function, and to explore their combination in mortality prediction in the early stage of sepsis. A total of 198 patients with bacterial infection and diagnosed with sepsis and 40 healthy control subjects were included. Blood samples were collected on admission within 24 h. Plasma concentrations of presepsin and PCT were measured. Expression of monocyte surface CD14, programmed cell death receptor ligand-1 (PD-L1) and human leucocyte Ag (HLA)-DR were determined using flow cytometry. Levels of plasma presepsin and PCT were significantly higher under septic conditions, and increased with the progression of sepsis. Monocyte CD14 and HLA-DR expression were decreased, while PD-L1 was overexpressed in sepsis compared to control. Presepsin and PCT concentrations were positively correlated with Sequential Organ Failure Assessment score, Acute Physiology and Chronic Health Evaluation System II score and PD-L1, while they were negatively correlated with CD14 and HLA-DR. Presepsin plus monocyte HLA-DR mean fluorescence intensity had the highest prognostic value over other parameters alone or in combination. Presepsin and PCT had a weak correlation with monocyte dysfunction during early sepsis. The combination of presepsin and monocyte HLA-DR could help improve prognostic value.

Keywords

Sepsis, presepsin, procalcitonin, monocyte, innate immunity, immune suppression

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Introduction

Sepsis and septic shock are major life-threatening health problems and potentially lead to more than 5.3 million annual deaths globally.¹ Sepsis is defined as a life-threatening organ dysfunction caused by a dysregulated host response to infection.² Increasing evidence has demonstrated the relationship between sepsis and dysregulation of the host immune system.

The innate immune system forms the first barrier to fight against invading pathogens. Recent studies have uncovered the suppression of the innate immune response during sepsis.^{3,4} Decreased expression of the antigen (Ag)-presenting molecule human leucocyte Ag (HLA)-DR on circulating monocytes is observed in

sepsis and is proposed as a surrogate marker of immune failure and poor prognosis.^{5,6} Overexpression of programmed cell death receptor ligand-1 (PD-L1)

¹Department of Emergency Medicine, Beijing Chaoyang Hospital, Capital Medical University, PR China

²Department of Emergency Medicine, Beijing Friendship Hospital, Capital Medical University, PR China

Corresponding author:

Chunsheng Li, Department of Emergency Medicine, Beijing Chaoyang Hospital, Capital Medical University, No. 8 Worker's Stadium South Road, Chaoyang District, 100020 Beijing, China.

Email: lcsyyy@163.com



occurs during sepsis and correlates with poor prognosis.^{7,8}

Monocytes play a vital role in the innate immune response. Monocyte surface Ag CD14 recognises LPS from bacteria, presents them to the TLR4-MD-2 complex and initiates the subsequent production of pro-inflammatory cytokines.⁹ Finally, a small soluble peptide structure is cleaved from the seminal CD14, namely soluble CD14 subtype (sCD14-ST) or presepsin.¹⁰ Multiple clinical studies have revealed that presepsin is efficient in differentiating infection, early diagnosis and risk stratification, and in predicting the prognosis of sepsis.^{11–13} Because of its close association with monocytes, presepsin has been highlighted as a novel predictor of innate immune responses during sepsis.^{14,15}

Procalcitonin (PCT) is a peptide with 116 amino acids, which is mainly secreted by C cells of thyroid neuroendocrine cells *in vivo*. After being stimulated by bacteria, the level of PCT will increase rapidly, showing a positive correlation with the degree of infection, and will be maintained for a certain period of time.¹⁶ PCT is not the initiator of the inflammatory cascade reaction in sepsis, but it can maintain and amplify the inflammatory response.¹⁷ PCT has a high specificity and sensitivity in the diagnosis of bacterial infection, and has a significant correlation with the severity of sepsis. Monitoring PCT levels during sepsis can reflect the effect of treatment.^{18,19} PCT has been widely used as a routine examination in the majority of emergencies for the early diagnosis of infection and sepsis.

Biomarkers such as presepsin, PCT and monocytes are all highly responsive when challenged by infection during early sepsis. Therefore, the aim of our study was to explore the possible relationship between the function of biomarkers and monocytes during early sepsis. In this investigation, the concentrations of plasma presepsin and PCT and the expression of CD14, HLA-DR and PD-L1 on circulating monocytes were measured. The association between biomarkers and monocytic surface Ag expression was explored. In addition, the prognostic value of presepsin, PCT and monocyte surface Ag alone or in combination in predicting sepsis were detected.

Methods

Study participants

This was an observational single-centre cohort study conducted in the Emergency Department (ED) of Beijing Chaoyang Hospital, a tertiary teaching hospital in China. This study was approved by the Medical Ethics Committee of Beijing Chaoyang Hospital.

Patients admitted to the ED (mainly from the Emergency Intensive Care Unit (EICU)) between October 2018 and August 2019 were enrolled according to the sepsis 3.0 criteria.² Patients who manifested with obvious clinical indications of bacterial infections, such as fever symptoms, elevated white blood cell or neutrophil counts and ground-glass opacity in chest X-rays, were enrolled. They were divided into sepsis or septic shock based on severity. Patients were excluded from the study if they (a) were < 18 years old; (b) received long-term hormone or immunosuppressive treatment; (c) were undergoing surgical trauma, blood-related diseases, liver diseases or end-stage renal failure; (d) had autoimmune or immunodeficiency diseases; (e) were diagnosed with cancer or were receiving chemotherapy or radiotherapy; or (f) were pregnant. All the patients underwent treatment based on the International Guidelines for the Management of Sepsis and Septic Shock (2016).²⁰ Healthy volunteers, matched by age and sex, who had never suffered from sepsis, hypertension, diabetes, or any major surgery were subsequently enlisted to be the control group. All subjects were followed up for 28 d, and 28 d mortality was defined as the primary end point.

Data and sample collection

Demographic files, clinical signs, laboratory test results and co-morbidities were recorded. The data collected were used to calculate the Acute Physiology and Chronic Health Evaluation System (APACHE) II score²¹ and the Sequential Organ Failure Assessment (SOFA) score.²² Data collection and subsequent experiments were accomplished by independent investigators who were blinded to grouping in order to avoid bias. Residual heparin anticoagulant venous blood (4 ml) from routine tests was collected on admission day within 24 h for flow cytometry. The blood was then centrifuged and the supernatant plasma was collected and stored at -80°C to determine the presepsin and PCT content.

Cell surface expression of biomarkers on monocytes

Circulating monocyte surface Ags CD14, HLA-DR and PD-L1 were measured within 3 h after blood collection by flow cytometry using a Gallios Flow Cytometer (Beckman Coulter, Brea, CA). The data were analysed using Gallios Software v1.0 (Beckman Coulter). The cells were stained, and the erythrocytes were subsequently lysed and washed according to the manufacturer's instructions. The following monoclonal Abs and their isotype controls, all obtained from BD Biosciences (San Jose, CA), were used per 100 μl of whole blood: Pacific Blue-labelled anti-CD14 (2 μl ;

cat. no. 558121), PerCP-Cy5.5-labelled HLA-DR (2 μ l; cat. no. 560652) and PE-labelled anti-CD274 (PD-L1; 10 μ l; cat. no. 557924). The flow cytometer calibration and protocol compensation were set up prior to the start of the experiment using BD CompBeads (BD Biosciences; cat. no. 552843). The threshold was defined using isotype controls (Pacific Blue isotype control, 2 μ l, cat. no. 558118; PerCP-Cy5.5 isotype control, 2 μ l, cat. no. 550927; APC isotype control, 10 μ l, cat. no. 555751; PE isotype control, 10 μ l, cat. no. 555749).

Monocytes were gated using CD14⁺ staining cells. The results are expressed as positive cell percentages and mean fluorescence intensity (MFI). Representative plots and the gating strategy are shown in Figure 1.

Measurements of plasma presepsin and PCT

Plasma presepsin concentrations were determined using a PATHFAST automated immunoanalyser (Mitsubishi Chemical Medicine Corp., Tokyo,

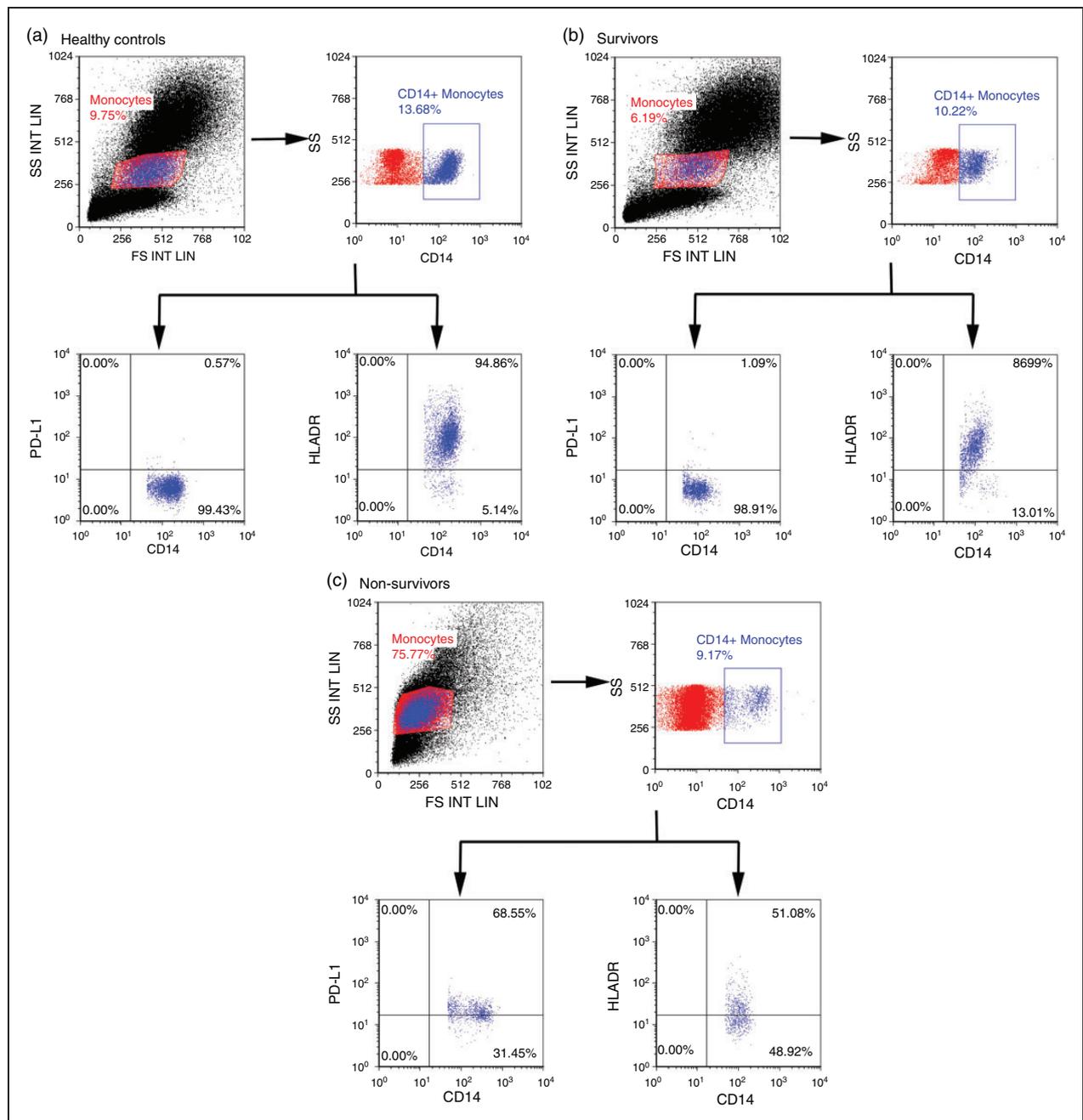


Figure 1. Representative flow cytometry plots and gating strategy. Monocytes were gated by CD14⁺ cells. HLA-DR and PD-L1 expression were measured on CD14⁺ staining gated monocytes of healthy controls (a), survivors (b) and non-survivors (c).

Japan). The upper and lower limits of detection were 20 and 200,000 pg/ml, respectively.

PCT concentrations were measured using a BioMerieux Mini VIDAS immunoassay analyser (Block Scientific, Bohemia, NY) within the detection range 0.05–200 ng/ml.

Statistical analysis

Normally distributed continuous variables are described as the mean \pm SD, and skewed distributions are described as the median (25th–75th percentiles). Quantitative data are expressed as frequencies and proportions. Comparisons between the groups were made using Pearson's chi-square test for categorical data and Mann–Whitney *U*-test for continuous variables. For multiple group comparison, the Kruskal–Wallis *H* test was applied. The correlation between two variables was analysed using the Spearman rank correlation test. The ability of presepsin and PCT to predict mortality was determined by the area under the curve (AUC) of the receiver operating characteristic (ROC) curve. Binary logistic regression was used to identify the variables resulting in 28-d mortality. Statistical significance was defined as a two-tailed *P* value of < 0.05 . All data and figures were processed using IBM SPSS Statistics for Windows v25.0 (IBM Corp., Armonk, NY) and MedCalc v19.0.7 (MedCalc Statistical Software, Mariakerke, Belgium).

Results

Baseline characteristics

A total of 198 patients with sepsis and 40 healthy volunteers were enlisted in this study. Based on severity, patients were segregated into those with sepsis ($n = 121$) and those with septic shock ($n = 77$). Based on the outcome on d 28, the subjects were also grouped into survivors ($n = 128$) and non-survivors ($n = 70$). The comparisons of baseline characteristics within the different groups are listed in Tables 1 and 2. No statistically significant difference was recorded between the group with sepsis and the healthy control group.

Changes of presepsin and PCT

As shown in Figure 2, the levels of presepsin and PCT were significantly elevated in the plasma samples of patients with sepsis compared to healthy control subjects ($P < 0.001$). Patients with septic shock had noticeably higher levels of plasma presepsin and PCT than patients with sepsis did ($P < 0.001$). Levels of presepsin and PCT were significantly higher in non-survivors than they were in those of survivors ($P < 0.05$).

Changes in monocyte surface Ag expression

As shown in Figure 3, expressions of CD14 and HLA-DR percentages were significantly lower and PD-L1

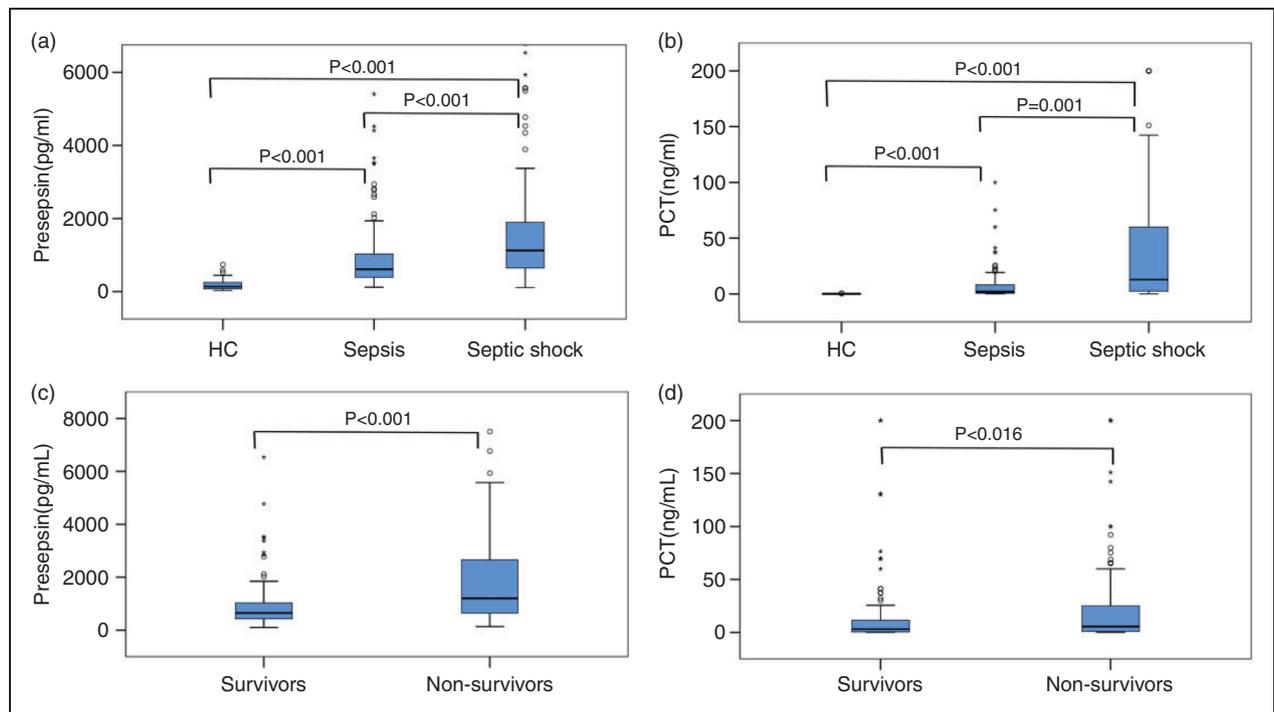
Table 1. Baseline characteristics of the participants.

Characteristics	Healthy control ($n = 40$)	Sepsis ($n = 121$)	Septic shock ($n = 77$)	<i>P</i> value
Age (yr), median (IQR)	68 (57–74)	72 (55–82)	70 (56–79)	0.425
Male/female	20/20	71/50	40/37	0.508
WBC count ($\times 10^9$)	5.81 (4.85–6.52)	13.02 (9.02–18.68)	11.74 (7.59–23.79)	< 0.001
Neutrophil percentage (%)	56.25 (48.03–63.33)	87.9 (81.95–91.50)	90.30 (81.55–94.05)	< 0.001
Neutrophil count ($\times 10^9$)	4.70 (3.62–5.65)	11.80 (7.87–16.93)	11.41 (6.54–20.90)	< 0.001
Lymphocyte percentage (%)	2.30 (1.70–2.70)	6.40 (3.45–11.05)	5.00 (3.15–12.70)	< 0.001
Lymphocyte count ($\times 10^9$)	2.12 (1.74–2.36)	0.82 (0.52–1.20)	0.65 (0.39–1.09)	< 0.001
Monocyte count ($\times 10^9$)	0.29 (0.27–0.38)	0.67 (0.38–1.00)	0.45 (0.19–0.74)	< 0.001
Lactate (mmol/l)	1.00 (0.60–1.38)	1.60 (1.15–2.25)	2.90 (1.72–6.70)	< 0.001
SOFA score	0 (0–1)	5 (3–7)	10 (8–12)	< 0.001
APACHE II score	1 (0–2)	13 (8–17)	19 (14–26)	< 0.001
28-d mortality, <i>n</i> (%)	0	30 (24.8%)	40 (51.9%)	< 0.001
Co-morbidity > 1 , <i>n</i> (%)	0	78 (64.5%)	62 (80.5%)	< 0.001
<i>Primary infection sites</i>				
Respiratory infection		68 (56.2%)	37 (48.1%)	0.263
Intra-abdominal infection		27 (22.3%)	20 (26.0%)	0.555
Cerebral infection		12 (9.9%)	7 (9.1%)	0.847
Urinary infection		11 (9.1%)	11 (14.3%)	0.257
Others		3 (2.5%)	2 (2.6%)	0.959

IQR: interquartile range; WBC: white blood cell; SOFA: Sequential Organ Failure Assessment; APACHE: Acute Physiology and Chronic Health Evaluation System.

Table 2. Baseline characteristics of septic patients on admission based on 28-d outcome.

Characteristics	Survivors (n = 128)	Non-survivors (n = 70)	Overall (n = 198)	P value
Age (yr), median (IQR)	70(58–80)	73(55–81)	71(56–80)	0.982
Male/female	67/61	44/26	111/87	0.362
WBC count ($\times 10^9$)	13.02 (8.31–19.16)	12.16 (7.75–19.91)	12.83 (8.00–19.28)	0.959
Neutrophil percentage (%)	87.8 n (81.30–92.15)	89.78 (83.15–93.40)	88.55 (81.98–92.75)	0.423
Neutrophil count ($\times 10^9$)	11.80 (7.25–17.23)	11.48 (6.93–17.96)	11.59 (7.08–17.69)	0.996
Lymphocyte percentage (%)	6.80 (3.25–11.40)	5.50 (3.35–10.55)	6.10 (3.30–11.13)	0.779
Lymphocyte count ($\times 10^9$)	0.84 (0.53–1.21)	0.68 (0.40–1.02)	0.77 (0.48–1.14)	0.393
Monocyte count ($\times 10^9$)	0.59 (0.34–0.99)	0.55 (0.21–0.90)	0.56 (0.28–0.98)	0.423
Lactate (mmol/l)	1.70 (1.15–2.45)	2.40 (1.60–4.75)	1.95 (1.30–3.23)	< 0.001
SOFA score	5 (3–7)	10 (7–12)	7 (4–10)	< 0.001
APACHE II score	12 (8–15)	20 (16–26)	15 (10–21)	< 0.001
Co-morbidity >1, n (%)	72 (56.3%)	61 (87.1%)	140 (70.7%)	< 0.001
Primary infection sites				
Respiratory infection	69 (53.9%)	36 (51.4%)	105 (53.0%)	0.946
Intra-abdominal infection	29 (22.7%)	18 (25.7%)	47 (23.7%)	0.890
Cerebral infection	12 (9.4%)	7 (10.0%)	19 (9.6%)	0.990
Urinary infection	14 (10.9%)	8 (11.4%)	22 (11.1%)	0.994
Others	4 (3.1%)	1 (1.4%)	5 (2.5%)	0.768

**Figure 2.** Comparisons of presepsin and PCT based on disease severity and outcome. Presepsin and PCT both increased significantly with the progression of sepsis (a, b). Presepsin and PCT were significantly increased in non-survivors than in survivors during sepsis (c, d).

percentage was significantly higher in patients with sepsis compared to controls ($P < 0.05$). Non-survivors had lower expression of CD14 ($P = 0.925$) and HLA-DR ($P < 0.05$) but slightly higher PD-L1 expression ($P = 0.876$) compared to survivors.

As shown in Figure 4, no significant differences were found in CD14 MFI between sepsis and controls. HLA-DR MFI significantly decreased with the severity of sepsis and was significantly lower in non-survivors than in survivors. PD-L1 MFI was only

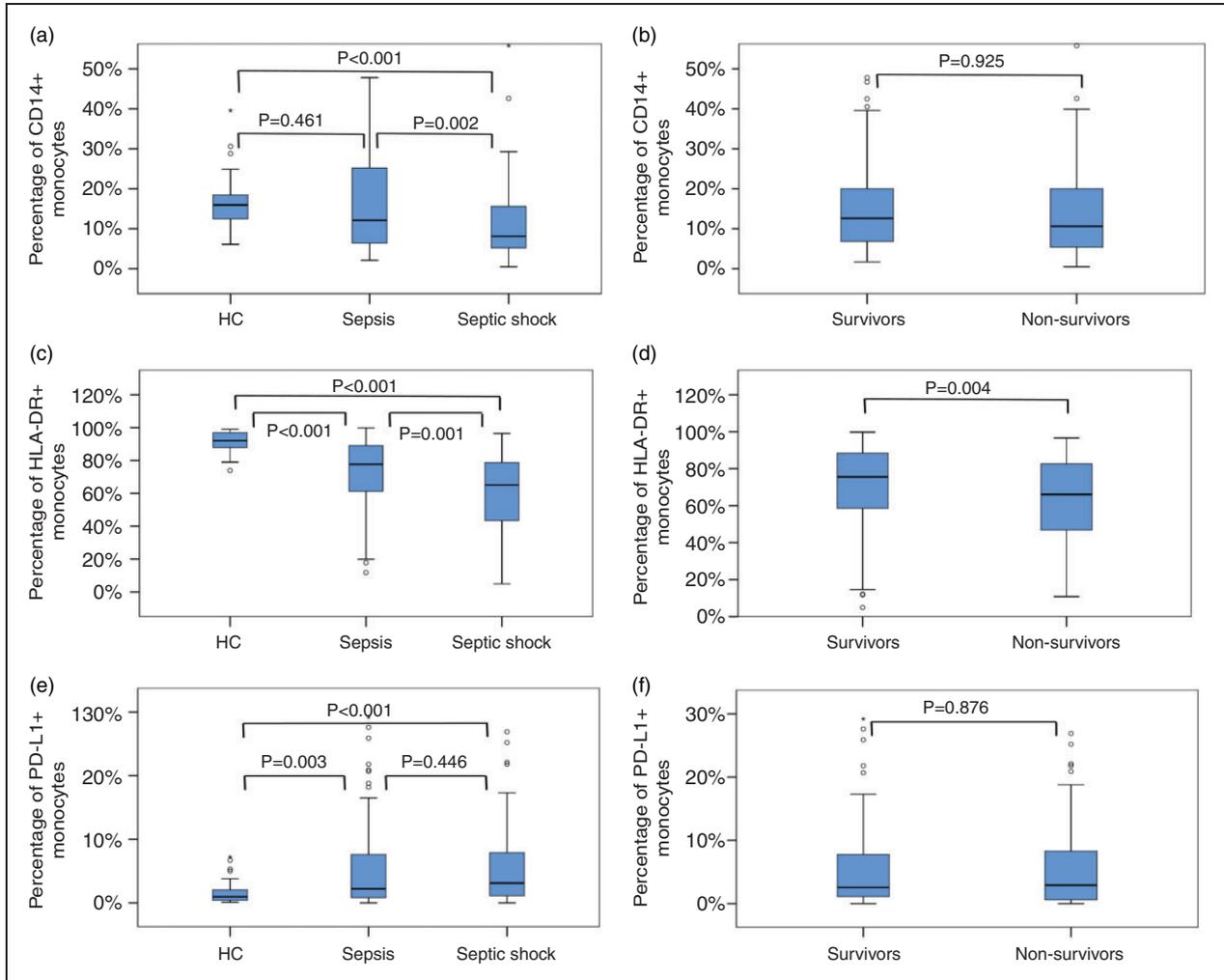


Figure 3. Comparisons of CD14, HLA-DR and PD-L1 positive cell percentages expression on monocytes based on severity and outcome. CD14 decreased as sepsis progressed (a, b). HLA-DR decreased significantly with the severity of sepsis (c, d) while PD-L1 increased as sepsis became severer (e, f).

significantly increased in septic shock compared to controls.

Correlation between presepsin, PCT and other parameters

The Spearman correlation analysis showed that plasma levels of presepsin were positively correlated with PCT, PD-L1 percentage, SOFA score and APACHE II score ($P < 0.05$; Figure 5). Moreover, presepsin were negatively correlated with CD14 percentage and HLA-DR percentage ($P < 0.05$). The same trend was observed in PCT (Figure 6).

The results of Spearman correlation analysis between presepsin, PCT and MFI expression of monocyte CD14, HLA-DR and PD-L1 are demonstrated in

Table 3. Only HLA-DR MFI was found to have a weak negative correlation with presepsin and PCT.

Prognostic value for presepsin and PCT

ROC curves were used to compare the prognostic value of presepsin and PCT. Data are presented in Figures 7 and 8 and Tables 4 and 5. The AUC for presepsin was significantly higher (0.699, $P < 0.001$) than that of PCT (0.599, $P < 0.001$) in predicting 28-d mortality ($Z = 2.179$, $P = 0.030$). As shown in Table 4, the AUC of presepsin plus monocyte CD14% had the highest prognostic value over other parameters alone or in combination (0.707, $P < 0.001$), but was not significantly higher than presepsin alone ($Z = 0.512$, $P = 0.608$). As shown in Table 5, the AUC of presepsin plus monocyte HLA-DR MFI had the highest

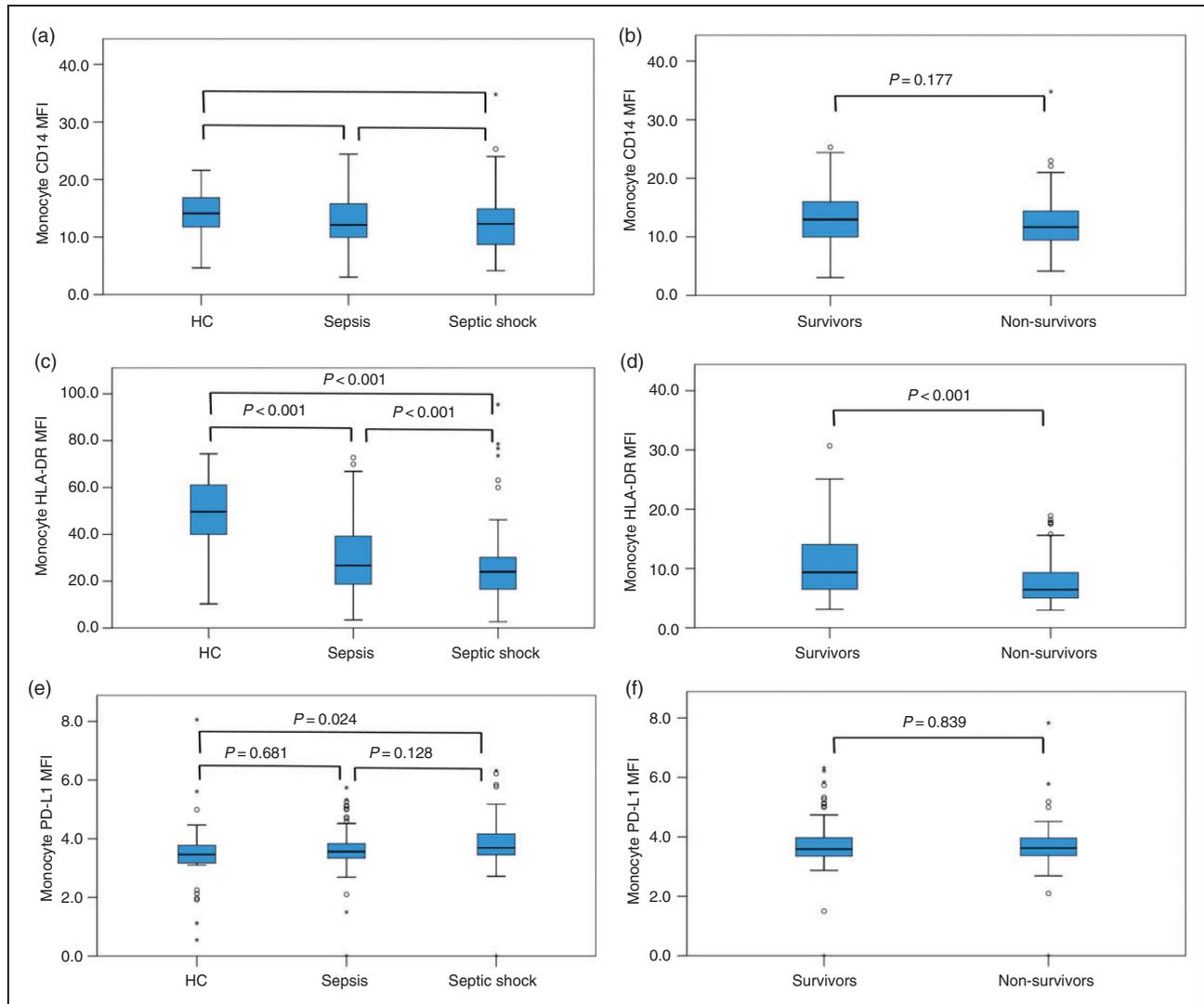


Figure 4. Comparisons of CD14, HLA-DR and PD-L1 MFI expression on monocytes based on severity and outcome. No significant differences were found in CD14 MFI between sepsis and controls (a, b). HLA-DR MFI significantly decreased with the severity of sepsis (c,d) while PD-L1 MFI was only significantly increased in septic shock compared with controls (e).

prognostic value over other parameters alone or in combination (0.727 , $P < 0.001$), and was also not significantly higher than presepsin alone ($Z = 0.794$, $P = 0.427$).

Independent risk factors for predicting 28-d mortality

We explored the independent risk factors that affect 28-d mortality using a multivariate Cox proportional hazards regression model for all the variances, including percentages and MFI of monocyte CD14, PD-L1, HLA-DR, PCT and presepsin. The statistical results are presented in Table 6. The results showed that monocyte HLA-DR MFI, PCT and presepsin were

the independent risk factors for predicting 28-d mortality in patients with sepsis ($P < 0.05$).

Discussion

In the present study, we found that plasma presepsin and PCT levels were significantly increased along with disease severity. Patients with poor outcome showed higher levels of presepsin, PCT and PD-L1 expression but lower levels of monocyte CD14 and HLA-DR expression in contrast to patients with a favourable outcome. Presepsin was negatively correlated with CD14 and HLA-DR but positively correlated with SOFA score, APACHE II score and PD-L1. A similar trend was observed with PCT. The combination of

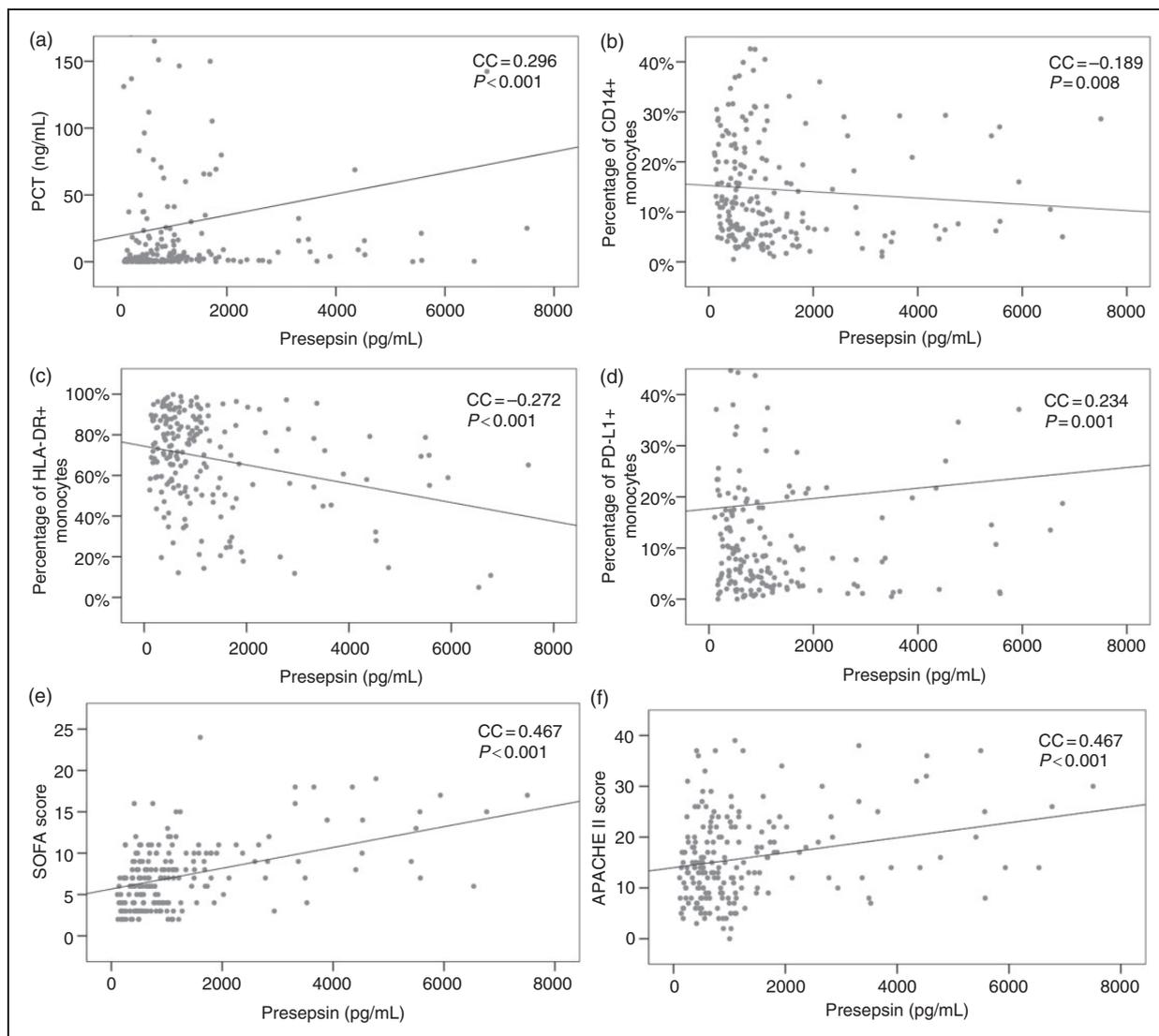


Figure 5. Correlation between prepsin and other parameters. Prepsin was significantly positively correlated with PCT, PD-L1, SOFA score and APACHE II score (a, d, e and f), while negatively correlated with CD14 and HLA-DR percentages (b, c).

prepsin and monocyte HLA-DR MFI had the highest value for predicting the mortality of sepsis. Furthermore, prepsin, PCT and HLA-DR MFI were independent predictors for the prognosis of sepsis. The results indicated that plasma biomarkers were related to the monocyte immune response of the host, and their combination could possibly help with the prognosis of sepsis.

Prepsin has been proposed as a novel biomarker during sepsis due to its quick detection and response to pathogens.²³ Consistent with previous studies,^{13,24} prepsin dramatically increased in early sepsis and was proportional to the severity and outcome. In non-survivors, the plasma levels of prepsin were significantly higher than in survivors. This was also

confirmed by the positive correlation between prepsin and the SOFA and APACHE II scores. This indicated that elevated levels of plasma prepsin predicted worse organ failure and physiological condition for sepsis.

Multiple studies have reported the application of prepsin versus PCT in the early diagnosis and prognosis of sepsis.^{11–13,24} In the present study, the AUC of prepsin in predicting mortality was significantly higher than that of PCT. Prepsin showed higher sensitivity, positive predictive value and negative predictive value than PCT did, supported by a previous cohort study conducted by Liu et al.¹³ This suggests that prepsin performs better than PCT in predicting prognosis during the early stages of sepsis, which could be helpful for initiating the appropriate therapy

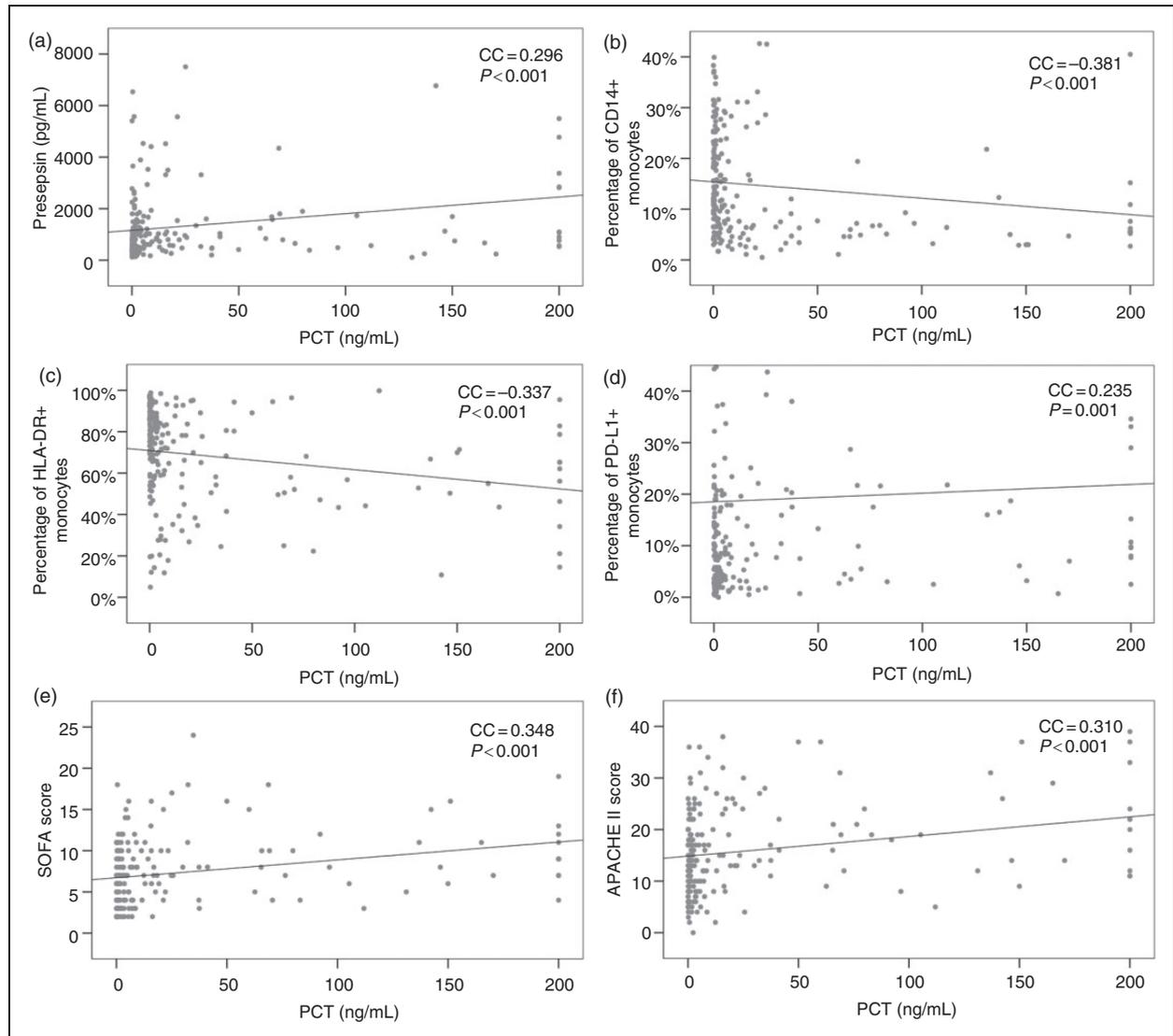


Figure 6. Correlation between PCT and other parameters. PCT was significantly positively correlated with presepsin, PD-L1, SOFA score and APACHE II score (a, d, e, and f), while negatively correlated with CD14 and HLA-DR percentages. (b, c).

Table 3. Correlations of presepsin, PCT and monocyte surface CD14, HLA-DR and PD-L1 MFI expression.

Correlations	Correlation coefficient	P value
Presepsin–monocyte CD14 MFI	−0.100	0.162
Presepsin–monocyte PD-L1 MFI	0.015	0.836
Presepsin–monocyte HLA-DR MFI	−0.258	< 0.001
PCT–monocyte CD14 MFI	−0.092	0.198
PCT–monocyte PD-L1 MFI	0.081	0.255
PCT–monocyte HLA-DR MFI	−0.363	< 0.001

PCT: procalcitonin. Significantly correlated *P*-values of less than 0.05 are marked in bold.

immediately. It should be noted that we enrolled patients using the sepsis 3.0 criteria, which was different from the criteria reported in previous studies. In addition, we selected critically ill septic patients mainly from the eICU, which means that the levels of PCT and presepsin should be higher than those in patients from the ED, resembling those of ICU patients.

Monocyte function forms an important part of innate immune response. From the observations of the present study, patients with worse conditions had significantly lower expression of monocyte CD14 and HLA-DR but overexpression of PD-L1. A reduction in CD14 expression represented the reduced ability of the body to initiate an immune response and induce the

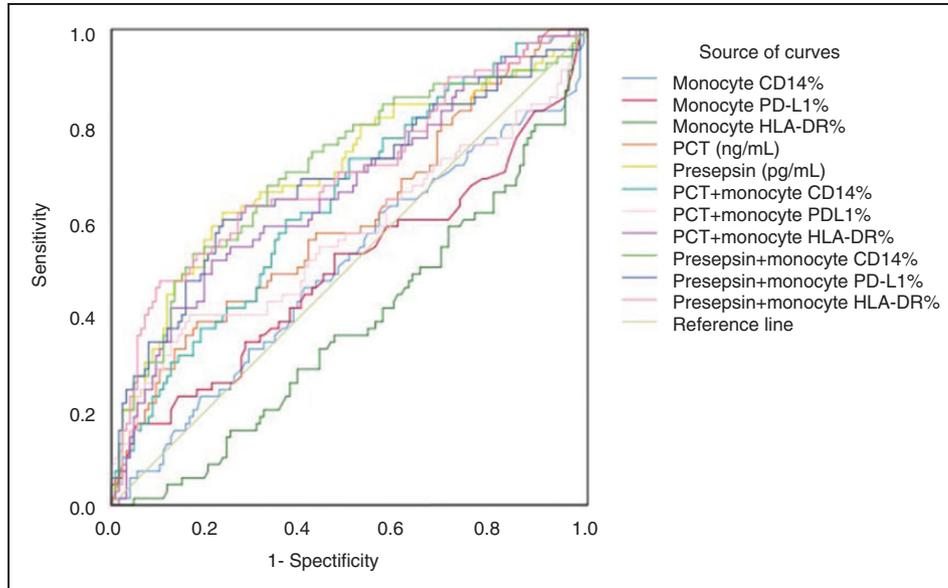


Figure 7. ROC curve of presepsin, PCT alone and in combination with monocyte CD14, PD-L1 and HLA-DR positive monocyte percentages for predicting 28-day mortality of sepsis.

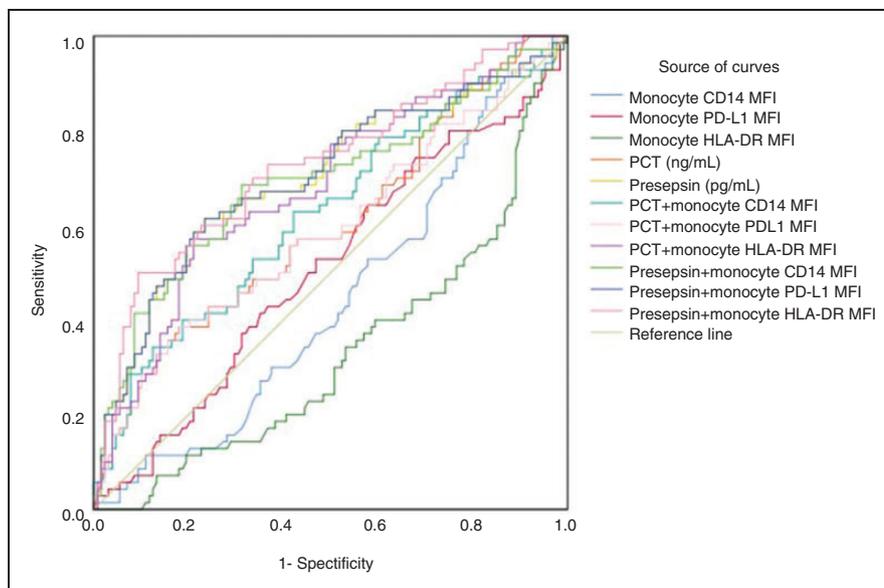


Figure 8. ROC curve of presepsin, PCT alone and in combination with monocyte CD14, PD-L1 and HLA-DR MFI on monocytes for predicting 28-day mortality of sepsis.

hyper-activation of phagocytes.^{25,26} Both monocyte HLA-DR percentage and MFI showed a significant reduction during sepsis and a weak correlation with presepsin and PCT. Besides, HLA-DR MFI was also an independent risk factor in predicting mortality. These indicate the importance of HLA-DR expression in reflecting disease severity. Decreased expression of HLA-DR on monocytes was associated with impaired Ag presentation and poor prognosis.^{5,6,9} In addition, a

weak trend of monocyte HLA-DR recovery was associated with secondary infection and poor prognosis.^{27,28} Moreover, monocyte PD-L1 overexpression was also recognised as an independent risk factor during septic shock.⁷ PD-L1 on monocytes could bind to PD-1 on effector T cells and suppress T-cell receptor signalling, leading to T-cell anergy, apoptosis and exhaustion.^{29–33} Thus, the results indicated that patients with sepsis had a dysregulated monocyte

Table 4. ROC curve parameters of presepsin, PCT alone and in combination with CD14, PD-L1 and HLA-DR positive monocyte percentages for predicting 28-d mortality in sepsis.

Parameters	AUC	SE	P value	95% confidence interval	
				Lower limit	Upper limit
Monocyte CD14%	0.496	0.044	0.925	0.409	0.582
Monocyte PD-L1%	0.493	0.046	0.876	0.404	0.583
Monocyte HLA-DR%	0.376	0.041	0.004	0.295	0.457
PCT (ng/ml)	0.599	0.043	0.021	0.515	0.683
Presepsin (pg/ml)	0.699	0.041	< 0.001	0.619	0.780
PCT + monocyte CD14%	0.642	0.041	0.001	0.562	0.721
PCT + monocyte PD-L1%	0.563	0.046	0.141	0.473	0.654
PCT + monocyte HLA-DR%	0.658	0.041	< 0.001	0.577	0.739
Presepsin + monocyte CD14%	0.707	0.040	< 0.001	0.628	0.786
Presepsin + monocyte PD-L1%	0.680	0.042	< 0.001	0.598	0.763
Presepsin + monocyte HLA-DR%	0.695	0.041	< 0.001	0.615	0.776

ROC: receiver operating characteristic; AUC: area under the curve. Statistically significant P-value of less than 0.05 predicting 28-d mortality in sepsis are marked in bold.

Table 5. ROC curve parameters of presepsin, PCT alone and in combination with monocyte CD14, PD-L1 and HLA-DR MFI for predicting 28-d mortality in sepsis.

Parameters	AUC	SE	P value	95% confidence interval	
				Lower limit	Upper limit
Monocyte CD14 MFI	0.442	0.042	0.177	0.360	0.524
Monocyte PD-L1 MFI	0.509	0.043	0.839	0.424	0.594
Monocyte HLA-DR MFI	0.332	0.040	< 0.001	0.253	0.411
PCT (ng/ml)	0.599	0.043	0.021	0.515	0.683
Presepsin (pg/ml)	0.699	0.041	< 0.001	0.619	0.780
PCT + monocyte CD14 MFI	0.627	0.042	0.003	0.544	0.710
PCT + monocyte PD-L1 MFI	0.590	0.044	0.037	0.504	0.676
PCT + monocyte HLA-DR MFI	0.682	0.040	< 0.001	0.603	0.761
Presepsin + monocyte CD14 MFI	0.691	0.042	< 0.001	0.609	0.773
Presepsin + monocyte PD-L1 MFI	0.701	0.041	< 0.001	0.621	0.782
Presepsin + monocyte HLA-DR MFI	0.727	0.039	< 0.001	0.651	0.803

Statistically significant P-value of less than 0.05 predicting 28-d mortality in sepsis are marked in bold.

Table 6. Cox regression model of independent risk factors for predicting 28-d mortality.

Parameters	B	SE	Wald	P-value	Exp(B)
Monocyte CD14 + %	0.015	0.011	1.775	0.183	1.015
Monocyte CD14 MFI	-0.007	0.022	0.102	0.749	0.993
Monocyte PD-L1%	0.014	0.012	1.394	0.238	1.015
Monocyte PD-L1 MFI	-0.014	0.127	0.012	0.912	0.986
Monocyte HLA-DR%	0.004	0.008	0.354	0.552	1.005
Monocyte HLA-DR MFI	-0.088	0.041	4.478	0.034	0.916
PCT (ng/ml)	0.004	0.002	4.582	0.032	1.004
Presepsin (pg/ml)	0.001	0.000	8.575	0.003	1.001

Parameters with P-value of less than 0.05 are considered as independent risk factors for predicting mortality in sepsis are marked in bold.

immune response, predisposing the host to a dangerous situation upon infection.

The changes in plasma biomarkers and monocyte surface Ag expression proved that pro-inflammatory and immunosuppression phases occur concomitantly during the early stages of sepsis.³⁴ Inflammation-induced organ injury, monocyte dysfunction and PD-L1 caused T-cell immunosuppression, which all contributed to poor prognosis and early mortality during sepsis.^{3,35} Additionally, patients who suffered from sepsis tended to be senile and had other chronic diseases or co-morbidities, which was also responsible for the immune status modification of circulating leucocytes in the initiation of sepsis.³⁶

Presepsin was generated directly from monocyte cleavage. Thus, it was considered to be an innate immune response biomarker.¹⁵ Therefore, it could be inferred that a relationship might exist between presepsin and immune-related Ag molecules on circulating monocytes. As expected, it was found that the concentration of presepsin was negatively correlated with those of monocytes CD14 and HLA-DR and positively correlated with PD-L1 expression under septic conditions, although the correlation was weak. During the early stages of sepsis, endocytosis of monocytes might diminish the abundance of CD14 on the plasma membrane, producing more secretory presepsin in the peripheral blood. Consequently, CD14 deficiency rendered cells poorly responsive to pathogens. Presepsin expression was associated with impaired monocyte function in its ability to recognise pathogens and present Ags in response to bacterial challenges. Plasma presepsin levels were also correlated with the PD-L1 pathway, which played a key role in the interaction with antigen presenting cells (APCs) with T cells in the pathogenesis of sepsis. PD-L1 expressed on monocytes and macrophages participated in the induction of T-cell exhaustion.³ The findings meant that monocyte dysfunction was associated with a small part of presepsin overproduction.

Surprisingly, the same trend was also observed in PCT. The PCT level had negative correlation with monocytes CD14 and HLA-DR but a positive correlation with PD-L1 expression, also to a weak extent. PCT is mainly secreted by C cells of thyroid neuroendocrine cells *in vivo* after bacteria stimulation.¹⁶ It seemed that PCT and monocytes had no direct relationship, which made the phenomena hard to explain. Further studies are needed to find out the mechanisms for the relationship between PCT and monocyte dysfunction. Based on our findings, PCT and presepsin could be used to stratify and evaluate the prognosis of sepsis as independent predictors. However, the overproduction of presepsin and PCT is not closely associated with impaired monocyte immunity, and could only reflect the host's

dysregulated innate immune status to a limited extent. Other factors might include interactions of other cytokines, immune cells, inhibitory molecules and so on, which need investigations in future studies.

In addition, because of the correlation of plasma biomarkers and monocyte dysfunction, it could be better to combine the two kinds of parameters in stratification and prognosis during sepsis. Interestingly, it was found that the combination of presepsin and monocyte HLA-DR MFI had the highest value in predicting death for sepsis over other parameters alone or in combination, although the two parameters only had a weak correlation. This result implied that including plasma biomarkers in combination with monocyte dysfunction parameters might help with optimising prognosis evaluation during sepsis.

There are several limitations that need to be pointed out in this study. Primarily, this was a single-centre cohort study, and the number of subjects was relatively small. A study should be conducted on a larger scale to verify these findings. Additionally, the effects of the chosen parameters were only observed over a short-term period, and the parameters were only measured at a single time point. An extended period of observation might help in better understanding the dynamic changes of the innate immune response to sepsis. Finally, due to the variances in cytometry, the reported results of monocyte surface Ags may differ using other instruments. Thus, it is important to establish a standardised protocol, such as monocyte HLA-DR measurements, to eliminate such potential errors.³⁷

Conclusion

Presepsin and PCT are both independent and useful biomarkers in the early stage of sepsis. Presepsin and PCT had a weak correlation with monocyte dysfunction in innate immunity. The combination of presepsin and monocyte HLA-DR MFI might improve prognostic value during sepsis.

Declaration of conflicting interests

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ORCID iD

Chunsheng Li  <https://orcid.org/0000-0002-3161-4457>

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