PROSPECTIVE COMPARISON OF NEUTROPHIL CD64, C-REACTIVE PROTEIN AND PROCALCITONIN IN THE IDENTIFICATION OF ICU SEPSIS-3

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ABSTRACT

Introduction*: In the middle income country China, we performed a prospective study aimed at evaluating the individual and combined diagnostic accuracy of PCT, CRP and neutrophil CD64 expression for differential diagnosis of sepsis-3 in critically ill patients at the time of ICU admission.*

Materials and methods*: We analyzed the CRP and PCT concentrations from 66 patients with sepsis and 24 non-septic ICU controls according to sepsis-3. In addition, CD64 on neutrophils was measured using quantitative flow cytometry.*

Results*: The sensitivity values of CD64, CRP and PCT were 77.27% (95% CI, 65.00–86.32), 87.88% (95% CI, 76.96-94.25) and 65.15% (95% CI, 52.34–76.19), respectively, and the specificity values were 91.67% (95% CI, 71.53-98.54), 58.33% (95% CI, 36.94-77.20) and 87.50% (95% CI, 66.54-96.71), respectively. The efficiency of various combinations of tests was also evaluated; the combination of PCT and CD64 in parallel testing balanced the sensitivity (84.85%) and specificity (83.33%) well and had the maximum Youden index (0.682).*

Conclusion*: Our data supported the potential of CD64, either alone or in combination with CRP/PCT, for routine clinical diagnosis of sepsis-3 in ICU populations in China.*

Keywords*: Sepsis-3, C-reactive protein, Procalcitonin, CD64, diagnosis.*

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Introduction

Sepsis, a syndrome of physiological, pathological and biochemical abnormalities induced by infection, is a major public health concern(1). The Intensive Care Over Nations (ICON) study has provided global epidemiologic data on 10,069 intensive care unit (ICU) patients, confirming that 2,973 (29.5%) of patients had sepsis on admission or during their ICU stay. The ICU and hospital mortality rates in patients with sepsis were 25.8% and 35.3%, respectively, which were significantly higher than the values in the general ICU population (ICU mortality, 16.2%; hospital mortality, 24.2%)[(2)].

Despite recent advances in medicine, sepsis remains one of the leading direct causes of death in ICUs[(3)]. The outcomes of sepsis have been shown to benefit from prompt diagnosis and early administration of appropriate antibiotic therapy[(3-5)], which can also prevent unnecessary, potentially harmful and costly therapeutic interventions for patients who do not have sepsis[(6)].

However, early diagnosis of sepsis still faces many challenges, because clinical signs of sepsis such as tachycardia, leucocytosis, tachypnea and pyrexia often overlap with those of other non-infectious conditions in critically ill patients[(7)]. Biomarkers are tools to assist physicians in

screening patients at risk of sepsis and then making decisions in clinical practice, especially in ICU populations. More than 170 biomarkers, including PCT and CRP, which are commonly used for laboratory diagnoses, have been studied for use in evaluation of sepsis[8]. Leukocyte surface molecules have been suggested to be promising possible markers of sepsis[9].

Flow cytometry is an innovative diagnostic method used in investigating sepsis. CD64, the high affinity immunoglobulin Fc γ receptor I, is constitutively expressed only by macrophages and monocytes. Its expression on neutrophils occurs after activation by cytokines such as interferon gamma (IFN-γ) and granulocyte colony-stimulating factor (G-CSF)[6]. Several studies have indicated that neutrophil CD64 is a specific and sensitive marker for the identification of sepsis in adult critically ill patients[3, 10, 11].

In 2016, sepsis-3 was newly defined as a life-threatening organ dysfunction caused by a dysregulated host response to infection. This definition emphasizes the primacy of the nonhomeostatic host response to infection, whose potential lethality is considerably greater than that of straightforward infection, as well as the need for urgent recognition(1). Although sepsis is a global priority, most available papers and evidence have disproportionately come from high income countries. In the middle income country China, we performed a prospective observational study aimed at evaluating the individual and combined diagnostic accuracy of PCT, CRP and neutrophil CD64 expression for differential diagnosis of sepsis-3 in critically ill patients at the time of ICU admission.

Materials and methods

Patient populations and blood samples

This single-center study was conducted in the ICU of the Affiliated Yantai Yuhuangding Hospital of Qingdao University from January 2016 to November 2017. Approval by the institutional review board and informed consent were obtained before patient inclusion. All patients were prospectively screened and recruited according to their presenting symptoms concerning sepsis within the first 24 h following admission. Sepsis in ICU treated patients was based on the presence of two or more qSOFA-criteria and suspected or confirmed infection. A control group comprised patients also in need of ICU treatment during the same period but with no immediate concern regarding sepsis. The exclusion criteria for all the patient groups were malignant tumors with metastases, hematological malignancy and the use of biological medication[11]. Demographic, clinical and biochemical data were collected throughout the ICU stays. The Acute Physiology and Chronic Health Evaluation (APACHE) score and the Sequential Organ Failure Assessment (SOFA) score was calculated at admission.

Laboratory measurements

The CRP and PCT measurements were analyzed through commercially available laboratory methods in our Laboratory Medicine department. The concentration of CRP in the serum was also determined with a BN™ II specific protein system (Siemens, Germany) according to the manufacturer's protocol. PCT concentrations were measured in serum samples, by using matched sandwich immunoassays (Elecsys BRAHMS PCT assay, Roche, Switzerland) and a electrochemiluminescence detection system (Cobas e411, Roche, Switzerland) according to the manufacturer's instructions. The peak value of CRP and PCT from days 0-2 in non-septic ICU patients was chosen.

CD64 index

The detection and analysis of the neutrophil CD64 index was performed according to the manufacturer's instructions, as described elsewhere(12-14). The expression of CD64 on neutrophils and monocytes was measured by quantitative flow cytometry with a BD FACS Canto (BD Biosciences, USA) instrument and Leuko64TM assays (Trillium Diagnostics, USA). The expression of CD64 on neutrophil surfaces was measured within 4 h after phlebotomy in EDTA-anticoagulated whole blood. Blood samples were prepared and analyzed according to the manufacturer's instructions. Monocytes and lymphocytes were used as internal positive and negative controls, respectively, and fluorescent beads were used for calibration. The intensity of CD64-expressed fluorescence was measured as the mean fluorescence intensity, as a linearized value on a log scale. Index calculations were performed in Leuko64 QuantiCalc software (Trillium Diagnostics, USA).

Statistical analysis

The normality of continuous variables was determined with the Kolmogorov-Smirnov test. Continuous variables are presented as mean (±SD)

or median (interquartile range (IQR)), and categorical variables are presented as absolute numbers with the corresponding proportion with 95% confidence intervals (CI). The significance of differences among groups was compared with the χ^2 test, Fisher exact test or Mann-Whitney U test, as appropriate. To evaluate the diagnostic performance biomarkers for sepsis, we estimated the area under the curve (AUC) of the receiver operating characteristic (ROC) curves, and the diagnostic accuracy for sepsis was compared with the DeLong test. The Youden index was used to identify the cutoff points. Performance parameters for each assay were evaluated by construction of 2 × 2 tables. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio and negative likelihood ratio of each assay were determined. Data were analyzed in SPSS Statistics 20 software (IBM, USA). A value of $p < 0.05$ was considered statistically significant.

Results

A total of 90 consecutive adult patients fulfilling the inclusion criteria (mean age: 61.66 ± 18.41 years) admitted to the ICU were enrolled, including 61 males (67.78%) and 29 females (32.22%). Definitions for diagnoses of ICU patients with sepsis, septic shock and no sepsis were made according to the third International Consensus Definitions for Sepsis and Septic Shock (sepsis-3)[1]. There were 66 patients categorized with sepsis (73.33%), of whom 24 had septic shock, and 24 non-septic patients (26.67%). Clinical details are provided in Supplemental Table S1.

A comparison of demographic and baseline data is shown in Table 1. No significant differences were seen in the age and sex distribution. Sepsis patients had higher white cell counts (WBC) in the ICU than non-septic ICU controls, but no significant APACHE score differences were found between the two groups. At admission, the CRP, PCT and CD64 index were higher in patients with sepsis than non-septic ICU controls ($p < 0.001$ for all the three markers) (Table 1). Moreover, we also observed differences in the CRP, PCT and CD64 index among the non-sepsis, sepsis and septic shock groups, as shown in Fig. 1. No statistically significant difference in CRP and CD64 was found between sepsis and septic shock patients. In contrast, the PCT level in septic shock ICU patients was higher in septic patients.

Specimen Information

Sepsis

Patient No.	Age	CD64[a]		CRP[a] (mg/L)		PCT[a] (μg/L)		WBC (10^9/L)	SOFA[b]	APACHE II[b]
1	68	7.01	+	347.0	+	100.000	+	25.49	13	30
2	65	2.83	+	93.7	+	0.791	—	11.75	8	15
3	65	3.02	+	9.8	—	2.160	+	11.09	12	25
4	43	5.30	+	481.0	+	11.560	+	24.71	5	5
5	96	2.28	+	91.0	+	0.446	—	20.35	9	26
6	79	3.42	+	231.0	+	5.460	+	6.79	6	14
7	47	0.95	—	119.0	+	1.710	—	8.33	8	18
8	72	8.77	+	142.0	+	0.770	—	16.06	10	32
9	86	0.51	—	14.4	—	0.400	—	6.00	10	39
10	28	0.67	—	14.2	—	0.509	—	9.04	4	15
11	79	1.78	—	94.6	+	1.470	—	11.09	8	22
12	68	1.59	—	68.0	+	0.881	—	13.89	6	24
13	62	2.22	+	398.0	+	7.070	+	20.79	5	19
14	78	4.23	+	288.0	+	100.000	+	11.03	8	21
15	67	4.54	+	132.0	+	2.120	—	4.79	7	23
16	88	2.16	+	222.0	+	1.600	—	15.70	7	27
17	81	5.52	+	50.0	+	100.000	+	16.60	15	24
18	29	1.60	—	67.4	+	82.870	+	22.73	10	28
19	30	2.86	+	163.0	+	4.020	+	21.37	6	14
20	41	0.76	—	70.5	+	3.150	+	19.96	11	23
21	80	3.24	+	141.0	+	9.580	+	15.73	7	26
22	67	1.90	—	100.0	+	2.740	+	28.03	9	20
23	39	3.43	+	56.0	+	0.020	—	10.34	6	8
24	58	3.23	+	132.6	+	17.260	+	16.98	8	14
25	57	2.22	+	50.4	+	0.146	—	11.14	7	19
26	52	2.34	+	34.4	+	0.665	—	25.24	16	19
27	48	2.45	+	374.0	+	2.450	+	14.40	9	16
28	85	1.29	—	131.0	+	4.940	+	7.52	10	21
29	25	7.83	+	208.0	+	6.490	+	37.62	3	15
30	49	2.45	+	50.0	+	1.240	—	12.07	10	17
31	45	0.78	—	5.0	—	0.738	—	5.53	7	6
32	76	0.74	—	42.8	+	0.110	—	11.89	12	16
33	77	5.10	+	229.0	+	41.650	+	13.21	7	17
34	57	3.40	+	80.8	+	0.684	—	11.27	9	13
35	73	18.64	+	200.0	+	19.400	+	6.58	7	37
36	50	3.18	+	11.7	—	99.620	+	12.66	7	28
37	53	7.77	+	157.0	+	30.230	+	9.93	11	20
38	51	7.65	+	222.0	+	100.000	+	3.81	3	19
39	80	3.46	+	8.2	—	1.070	—	14.74	20	55
40	38	1.53	—	142.0	+	0.737	—	7.50	9	13
41	41	6.53	+	143.0	+	16.850	+	3.29	12	16
42	62	1.69	—	224.0	+	0.672	—	9.71	9	30
43	76	7.14	+	200.0	+	100.000	+	3.71	12	25
44	60	9.69	+	409.0	+	4.890	+	10.09	9	17
45	63	6.00	+	454.0	+	66.470	+	18.14	4	17
46	26	3.30	+	104.7	+	1.010	—	8.90	4	4
47	74	3.73	+	200.0	+	20.920	+	24.91	6	20
48	72	3.65	+	188.8	+	59.590	+	23.81	5	18
49	54	1.66	—	33.5	+	1.520	—	12.38	12	11
50	69	9.40	+	87.4	+	21.520	+	19.12	7	19
51	65	3.29	+	139.0	+	3.380	+	22.60	20	36
52	68	4.01	+	145.0	+	34.170	+	14.50	13	25
53	46	3.94	+	370.0	+	6.400	+	12.28	8	23
54	65	1.61	—	47.6	+	3.430	+	25.46	9	30
55	54	5.67	+	182.5	+	100.000	+	5.60	10	22
56	70	6.34	+	339.0	+	100.000	+	10.55	9	19
57	34	6.56	+	301.0	+	77.190	+	15.40	7	13
58	46	10.62	+	129.0	+	0.132	—	11.24	7	22
59	49	9.48	+	72.3	+	5.310	+	8.33	9	22
60	64	4.24	+	198.0	+	100.000	+	8.46	8	24
61	61	4.18	+	261.0	+	3.200	+	10.89	8	12
62	80	9.16	+	316.0	+	11.130	+	6.43	8	15
63	82	6.05	+	20.6	—	46.480	+	9.87	12	22
64	68	4.74	+	189.0	+	49.270	+	3.04	13	18
65	84	5.05	+	288.0	+	18.740	+	4.18	12	32
66	54	2.19	+	3.1	—	7.590	+	10.94	10	21

[a]The cut-off is 2.155 for CD64 index, 33.45 mg/L for CRP and 2.145μg/L for PCT, respectively.
[b]SOFA, Sequential Organ Failure Assessment; APACHE II, Acute Physiology and Chronic Health Evaluation (APACHE) score II

Table S1

Non-sepsis

Patient No.	Age	CD64		CRP(mg/L)		PCT(μg/L)		WB-C(10^9/L)	APACHE II
1	92	1.07	—	4.7	—	0.968	—	3.66	10
2	86	0.54	—	24.3	—	0.037	—	8.95	37
3	70	2.49	+	27.7	—	38.580	+	10.20	27
4	65	1.96	—	33.4	—	10.340	+	7.52	12
5	69	1.40	—	154.0	+	0.537	—	17.78	16
6	97	1.29	—	90.5	+	1.290	—	9.53	17
7	69	1.55	—	51.2	+	3.070	+	20.43	14
8	72	0.31	—	135.0	+	0.106	—	16.05	25
9	95	0.43	—	5.0	—	0.430	—	8.60	18
10	84	0.57	—	102.0	+	1.630	—	17.50	23
11	31	0.55	—	3.0	—	0.020	—	5.70	8
12	54	2.15	—	308.9	+	0.322	—	7.91	10
13	69	0.41	—	3.0	—	0.049	—	4.37	12
14	55	0.61	—	24.9	—	0.085	—	13.79	20
15	78	2.38	+	50.8	+	0.141	—	5.73	29
16	65	0.96	—	11.7	—	1.190	—	7.24	12
17	16	0.46	—	3.2	—	2.130	—	13.70	8
18	15	0.75	—	3.2	—	0.053	—	6.50	3
19	45	0.98	—	130.0	+	1.320	—	7.92	19
20	44	0.85	—	4.7	—	0.895	—	11.92	29
21	74	1.22	—	100.0	+	0.584	—	7.41	11
22	63	0.25	—	8.0	—	0.118	—	4.83	10
23	47	0.96	—	12.2	—	0.069	—	16.04	22
24	75	1.71	—	168.0	+	1.210	—	11.06	25

Table S1

	Non-sepsis	Sepsis	*p*
Age (years)	63.75 ± 22.20	60.89 ± 16.95	0.518
Gender, male/female	16/8	45/21	0.892
WBC	10.18 ± 4.71	13.45 ± 7.08	0.039
APACHE II	17.38 ± 8.376	20.85 ± 8.336	0.496
CRP (mg/L) (median [IQR])	26.3 (96.7)	140 (158.0)	<0.001
PCT (μg/L) (median [IQR])	0.561 (1.222)	5.125 (34.985)	<0.001
CD64 index (median [IQR])	0.96 (0.97)	3.43 (3.83)	<0.001

Table 1: Characteristics of the study population.

higher than those for CRP (P = 0.020) and PCT (P = 0.040). The AUC values for CRP and LBP were similar (P = 0.630).

The results of CRP, PCT and CD64 tests are shown in Table 2. For each assay, diagnostic parameters such as sensitivity, specificity, positive/negative likelihood ratio, positive/negative predictive value and Youden index were calculated (Table 2). A comparison of the three assays showed that CRP had the highest sensitivity (87.88%, 95% CI, 76.96-94.25), whereas CD64 was the most specific marker (91.67%, 95% CI, 71.53-98.54). In addition, CD64 showed the best performance in positive likelihood ratio, PPV and Youden index. CRP was the most optimal marker in negative likelihood ratio and NPV.

Comparison of the performance of biomarker combinations

To determine the optimal diagnostic strategy, we compared the performance of various combinations of the three markers involved in this study (Table 3). Parallel testing (any one positive is positive), which is opposite from serial testing (any one negative is negative), may increase sensitivity but decrease specificity accordingly. The most optimal sensitivity (95.45%, 95% CI, 86.44-98.82), negative likelihood ratio and NPV were achieved by combining CRP and CD64 in serial testing. A combination of CRP and PCT in parallel testing appeared to be better than other combinations in terms of specificity, positive likelihood ratio and PPV.

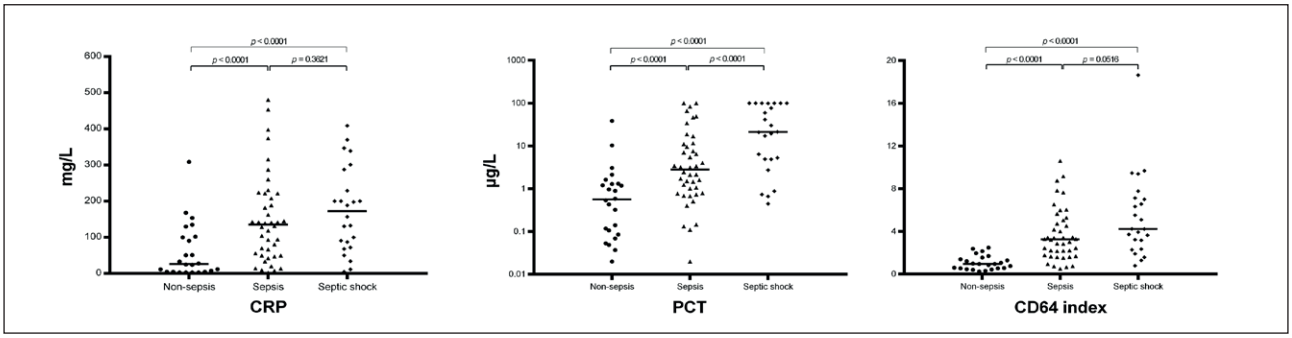

Figure 1: Overview of the parameters tested in non-sepsis, sepsis and septic shock patients.

Comparison of the performance of individual assays

ROC curve (Fig. 2) analysis revealed AUC values of 0.780 (95% CI: 0.672-0.888, p < 0.001) for CRP, 0.814 (95% CI: 0.718-0.910, p < 0.001) for PCT and 0.905 (95% CI: 0.845-0.965; p < 0.001) for CD64 to identify patients with sepsis, respectively. The AUC value for CD64 was significantly

Discussion

Recent large-scale epidemiological studies have shown that the mortality rate of sepsis has decreased, but its incidence continues to increase[15]. Sepsis continues to be a major health problem and a leading cause of death worldwide, especially in the ICU setting[16]. The World Health Assembly and

WHO made sepsis a global health priority in 2017 and have adopted a resolution to improve the prevention, diagnosis and management of sepsis[17].

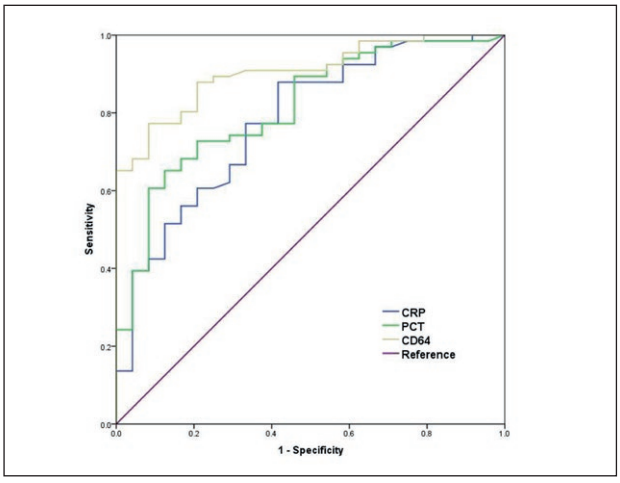

Figure 2: Overview of the parameters tested in non-sepsis, sepsis and septic shock patients.

Parameter*	Biomarker		
	CRP	PCT	CD64
cut-off	33.45 mg/L	2.145μg/L	2.155
Sensitivity % (95% CI)	**87.88 (76.96-94.25)**	65.15 (52.34-76.19)	77.27 (65.00-86.32)
Specificity% (95% CI)	58.33 (36.94-77.20)	87.50 (66.54-96.71)	**91.67 (71.53-98.54)**
PLR (95% CI)	2.11 (1.30-3.41)	5.21 (1.78-15.24)	**9.27 (2.44-35.18)**
NLR (95% CI)	**0.21 (0.10-0.42)**	0.40 (0.28-0.56)	0.25 (0.16-0.39)
PPV% (95% CI)	85.29 (74.15-92.35)	93.48 (81.07-98.30)	**96.23 (85.92-99.34)**
NPV% (95% CI)	**63.64 (40.83-81.97)**	47.73 (32.73-63.12)	59.46 (42.19-74.80)
Youden	0.462	0.527	**0.690**

Table 2: Clinical performance of biomarkers in diagnosing sepsis-3.
**Best performance parameters are shown in bold. PLR = positive likelihood ratio, NLR = negative likelihood ratio,* PPV = positive predictive value, NPV = negative predictive value.

Nevertheless, most of the available papers and evidence have come disproportionately from high income countries. It is also important for the global research and quality-improvement agendas on sepsis not to neglect low income and middle income countries[18]. We performed this study in the middle-income country China, to evaluate the diagnostic accuracy of PCT, CRP and CD64 in identifying sepsis-3 in critically ill patients at ICU admission.

We found that the median values of CD64, CRP and PCT were significantly higher in patients with sepsis-3 than in the non-septic control group, a result in agreement with previous data[11]. Higher PCT levels have been associated with increased mortality rates and correlated with severity scores associated with the severity of sepsis[6, 19, 20].

Our present results also indicated that the PCT levels were higher in septic shock ICU patients than septic patients, but both CRP and CD64 showed few differences between the septic shock and sepsis group. PCT has also drawn attention because it can be used for guidance in antibiotic treatment, to reduce inappropriate use of antibiotics[21]. In addition, we determined the optimal thresholds of the three markers for diagnosing sepsis-3 (Table 2).

Our results suggested that CD64 was the most specific marker (91.67% vs 58.33% vs 87.50%) and further supported that CD64 was suitable for confirming sepsis cases or excluding suspicious cases, because microbiologic body fluid cultures required at least 24-48 h, and negative cultures cannot exclude sepsis. These findings may have potential implications for vulnerable patients, because unreasonable use of antibiotics may lead to the emergence of adverse events, medical complications or worse conditions.

Parameter*	Biomarker combination†					
	CRP or PCT	CRP and PCT	CRP or CD64	CRP and CD64	PCT or CD64	PCT and CD64
Sensitivity %(95% CI)	93.94 (84.44-98.04)	59.09 (46.30-70.82)	**95.45 (86.44-98.82)**	69.70 (57.00-80.09)	84.85 (73.44-92.11)	56.06 (43.35-68.07)
Specificity%(95% CI)	50.00 (29.65-70.35)	**95.83 (76.88-99.78)**	54.17 (33.24-73.83)	**95.83 (76.88-99.78)**	83.33 (61.81-94.52)	**95.83 (76.88-99.78)**
PLR	1.88 (1.25-2.82)	14.18 (2.06-97.62)	2.08 (1.34-3.23)	**16.73 (2.44-114.70)**	5.09 (2.07-12.53)	13.45 (1.95-92.75)
NLR	0.12 (0.04-0.34)	0.43(0.32-0.57)	**0.08 (0.03-0.27)**	0.32 (0.22-0.46)	0.18 (0.10-0.33)	0.46 (0.35-0.60)
PPV%(95% CI)	83.78 (72.99-90.98)	97.50 (85.27-99.87)	85.14 (74.53-91.99)	**97.87 (87.28-99.89)**	93.33 (82.99-97.84)	97.37 (84.57-99.86)
NPV%(95% CI)	75.00 (47.41-91.67)	46.00 (32.06-60.55)	**81.25 (53.69-95.03)**	53.49 (37.83-68.53)	66.67 (47.14-82.06)	44.23 (30.73-58.58)
Youden	0.439	0.549	0.496	0.655	**0.682**	0.519

Table 3: Clinical performance of biomarker combinations in diagnosing sepsis-3.
**Best performance parameters of test combinations are shown in bold. †In parallel testing (including CRP or PCT, CRP or CD64, and PCT or CD64), a patient was considered positive when either test was positive. In serial testing (including CRP and PCT, CRP and CD64, and PCT and CD64), a patient was considered positive only when both tests were positive.*

Some previous studies have summarized the ability of CD64 expressed on neutrocytes to discriminate between infected and non-infected critically ill patients and have consistently found good diagnostic performance[6, 10, 11].

The sensitivity and specificity of CD64 in our study were in accordance with findings from a recent meta-analysis by Xiao Wang et al., which incorporated eight studies and found a mean sensitivity and specificity of 76% (95% CI, 73-78%) and 85% (95% CI, 83-86%), respectively[22], and another study by Joan Cid et al., which incorporated 13 studies and found a mean sensitivity and specificity of 79% (95% CI, 70-86%) and 91% (95% CI, 85–95%), respectively[23]. However, the sensitivity of CD64 in this study appeared to be inconsistent with those reported by Aikaterini Dimoula (89%), Joel Jämsä (100%) and Shudao Xiong (93.9%) et al.[6, 11, 24]. Some differences in study design between these previous studies and the present study may explain the conflicting results. First, sepsis was defined according to the third International Consensus Definitions for Sepsis and Septic Shock in our study, whereas the sensitivity in the previous studies was determined by the ability to differentiate the sepsis-2 from the systemic inflammatory response syndrome (SIRS). In addition, diagnostic performance may vary among races and clinical settings. Gros et al. have criticized the low sensitivity of neutrophil CD64 in sepsis diagnostics in the ICU patients, but this method may be useful in combination with a more sensitive biological marker, given its high specificity[25].

The present data confirmed that CRP was more sensitive than PCT and CD64 (87.88% vs 65.15% vs 77.27%). CD64 may therefore have utility in critical care when it is used in conjunction with another highly sensitive marker such as CRP, which lacks specificity. In general, our findings were consistent with previous reports of the excellent performance of CD64 in sepsis detection, because the AUC (0.905, 95% CI: 0.845-0.965) was significantly higher than those for CRP and PCT (Fig. 2). However, the use of CD64 in everyday practice may be limited by the cost of round-the-clock flow cytometric facilities.

Our present results indicated that no single marker was sufficient for precise diagnostics. A combination of biomarkers may offer different strategies to improve diagnosis and treatment efficacy, and consequently patient outcome. A combination of CRP and CD64 in parallel testing may be more suitable for screening suspicious septic patients, owing to the higher sensitivity (95.45%). In contrast, the specificity increased to 95.83% through use of a combination of any two of the three biomarkers as serial tests; this method is thus useful for excluding suspicious sepsis and decreasing suffering and expenses for weak patients. Moreover, the combination of PCT and CD64 in parallel testing balanced the sensitivity (84.85%) and specificity (83.33%) well and had the maximum Youden index (0.682), thus suggesting good potential for routine clinical diagnosis. Because sepsis is a tremendously complex and heterogeneous biological syndrome, a combination of biomarkers is more likely to account for this heterogeneity than a single biomarker[26]. The combination of the three biomarkers should improve diagnostic accuracy and may be particularly useful in specific clinical situations.

Several limitations of our study should be addressed. Because this was a single-center study with a small sample size in the middle income country China, the results may not be applicable to other settings or different populations. Validation in large multicenter studies may be needed to definitely determine whether neutrophil CD64 can be recommended as a sepsis-3 biomarker for routine use.

In conclusion, our prospective comparisons suggested that the median values of CD64, CRP and PCT were significantly higher in sepsis-3 patients than non-septic ICU patients. CD64 showed excellent diagnostic performance in sepsis-3, with its AUC of (0.905, 95% CI: 0.845-0.965), and appeared to be a promising tool for routine diagnosis of sepsis-3. Combinations of biomarkers may have the best potential to provide highly sensitive and specific real-time results to influence bedside diagnostic and therapeutic decisions.

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