Received: 10 February 2022 | Revised: 16 April 2022 | Accepted: 29 April 2022

DOI: 10.1002/jcla.24505

RESEARCH ARTICLE

WILEY

Combination of C-reactive protein, procalcitonin, IL-6, IL-8, and IL-10 for early diagnosis of hyperinflammatory state and organ dysfunction in pediatric sepsis

Gongbo Zeng | Dong Chen | Renxi Zhou | Xinfeng Zhao | Cuiying Ye | Huiting Tao | Wenbin Sheng | Yidong Wu

Hangzhou Children's Hospital, Hangzhou, China

Correspondence
Yidong Wu, Hangzhou Children's Hospital, Hangzhou, 195 Wenhui Road, 310014, China.
Email: wyd721@sina.com

Funding information
The Science and Technology Plan Guidance Project of Hangzhou, Grant/Award Number: 20201231Y051 and 20191231Y049; The Municipal Key Discipline of Hangzhou of Respiratory Diseases and Infectious Diseases in Children

Abstract

Background: Although early diagnosis and management are critical for prognosis of pediatric sepsis, there are no specific diagnostic biomarkers for the hyperinflammatory state and organ dysfunction, important stages of sepsis.

Methods: We enrolled 129 children with infection into three groups: non-sepsis infection (33), Sepsis 1.0 (hyperinflammatory state, 67), and Sepsis 3.0 (organ dysfunction, 29). Another 32 children with no infections were included as controls. Serum C-reactive protein (CRP), procalcitonin (PCT), interleukin (IL)-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IL-17, tumor necrosis factor (TNF)-α, interferon (IFN)-α, and IFN-γ were assessed to diagnose the two stages, and their diagnostic capacities were evaluated using receiver operating characteristic (ROC) curves. We also examined whether combining biomarkers improved diagnostic efficiency.

Results: Significantly higher CRP, PCT, and IL-6 levels were detected in the Sepsis 1.0 than the non-sepsis infection group ($p < 0.001$). The areas under the curve (AUCs) for diagnosing Sepsis 1.0 were 0.974 (CRP), 0.913 (PCT) and 0.919 (IL-6). A combination of any two biomarkers increased diagnostic sensitivity to ≥92.54% and specificity to 100.00%. Significantly higher PCT, IL-8, and IL-10 levels were found in the Sepsis 3.0 than the Sepsis 1.0 group ($p \leq 0.01$), with AUCs for diagnosing Sepsis 3.0 0.807 (PCT), 0.711 (IL-8), and 0.860 (IL-10). Combining these three biomarkers increased diagnostic sensitivity to 96.55% and specificity to 94.03%.

Conclusion: In pediatric sepsis, combining any two of CRP, PCT, and IL-6 can accurately diagnose the hyperinflammatory state and increase diagnostic specificity. Early diagnosis of organ dysfunction requires a combination of PCT, IL-8, and IL-10.

KEYWORDS
C-reactive protein, cytokines, hyperinflammatory state, organ dysfunction, pediatric sepsis, procalcitonin

Gongbo Zeng and Dong Chen contributed equally to this work and should be considered to share first authorship.

1 | INTRODUCTION

Sepsis was previously defined as systemic inflammatory response syndrome (SIRS) caused by infection, with severe sepsis defined as sepsis with organ dysfunction.[1,2] However, these definitions only emphasized the excessive inflammation without reflecting the host response to infection.[3] Further research showed that the essence of sepsis is the dysregulated response caused by infection. The early hyperinflammatory state, known as SIRS, with the later compensatory anti-inflammatory response syndrome (CARS) together form the dysregulated inflammation, which is reflected by organ dysfunction.[3–5] Therefore, in 2016, the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3) modified the definition of sepsis as life-threatening organ dysfunction caused by a dysregulated host response to infection.[3] A Sequential Organ Failure Assessment (SOFA) score of ≥2 (with a mortality rate of approximately 10%) was used as the clinical judgment standard for organ dysfunction.[3] In 2017, Matics and Sanchez-Pinto applied Sepsis-3 in children, proposing the Pediatric Sequential Organ Failure Assessment (pSOFA).[6] Although the 2005 Definition for Sepsis and Organ Dysfunction in Pediatrics description of pediatric sepsis as SIRS caused by infection is still applicable,[7] it is increasingly clear that infection-induced host-dysregulated response and organ dysfunction play an essential role in the diagnosis.[8,9]

Although etiological evidence is necessary for diagnosis, the process can be slow, with a low positivity rate.[10,11] Early diagnosis plays a crucial role in the prognosis of sepsis.[12] Many biomarkers have been used clinically to assist the sepsis diagnosis, including C-reactive protein (CRP), procalcitonin (PCT), interleukin (IL)-6, IL-8, and IL-10.[13–15] However, specific biomarkers for the hyperinflammatory state or dysregulated inflammation causing organ dysfunction have not been established.

In this study, we retrospectively analyzed acute phase levels of CRP, PCT and the cytokines IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IL-17, tumor necrosis factor (TNF)-α, interferon (IFN)-α, and IFN-γ in serum samples from children diagnosed with infection or sepsis. We assessed the role of these biomarkers in early diagnosis of the two important stages in pediatric sepsis, the hyperinflammatory state and organ dysfunction resulting from dysregulated inflammation, and tried to improve the diagnostic efficiency by combining these biomarkers. Finally, we evaluated the best combination of biomarkers to more accurately diagnose both the hyperinflammatory state and organ dysfunction to facilitate early intervention and improve the prognosis of pediatric sepsis patients.

2 | MATERIALS AND METHODS

2.1 | Study population and reference standard

A retrospective analysis of children treated in Hangzhou Children's Hospital between March 2019 and November 2021 was performed. As controls, children with non-infectious diseases, no SIRS and with a pSOFA score of <2 were included in the no infection group. There were three case groups, as follows. Children with an infection, no SIRS, and a pSOFA score of <2 were included in the non-sepsis infection group. Children with an infection, SIRS, and a pSOFA score of <2 were categorized as Sepsis 1.0, representing the hyperinflammatory state. Children with an infection and a pSOFA score of ≥2 were categorized as Sepsis 3.0, representing the dysregulated inflammation phase of sepsis that results in organ dysfunction. Details of the criteria for SIRS[7] and the pSOFA score[6] used in this study are summarized in the Table S1 and S2. In brief, a diagnosis of SIRS was based on acute phase temperature, leukocyte count, heart rate, respiratory rate, and systolic blood pressure. Six items were assessed to obtain a pSOFA score, including respiratory function (evaluated either by the partial pressure of oxygen/fraction of inspired oxygen (PaO_2/FiO_2) or the peripheral oxygen saturation/fraction of inspired oxygen (SpO_2/FiO_2)), mean arterial pressure (MAP), platelet count (PLT), serum total bilirubin (TBIL), serum creatinine (CREA), and the Glasgow Coma Score. Exclusion criteria for this study included neonates (0–28 days old) and children with other conditions that could interfere with the pSOFA score. This study was approved by the Ethics Committee of Hangzhou Children's Hospital. (File No: 2021 Ethics Review [Clinical Research] No. 91).

2.2 | Sample measurements and data collection

All the children's clinical data and laboratory samples were collected from the acute phase. Serum CRP levels were determined using latex-enhanced immunoturbidimetry; leukocyte and platelet counts were determined using the flow cytometric impedance method; all these assays were performed using a BC-5100-CRP automatic blood analysis system (Mindray). PCT was quantified by electrochemiluminescence in an E602 automated immunoassay analyzer (Roche). CREA was assessed using an enzymatic method (SEKISUI), and vanadate oxidation (FUJIFILM, Japan) was used to quantify TBIL; both assays were performed in a HITACHI 7600 automatic analyzer (HITACHI). Serum cytokine levels (IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IL-17, TNF-α, IFN-α, and IFN-γ) were quantitatively determined using cytokine 12-item kits (Raisecare) in a NAVIO flow cytometer (Beckman Coulter). All samples were stored and tested according to the assay manufacturers' instructions. The upper limit of the normal reference range for each biomarker was as following: CRP, 10.00 mg/L; PCT, 0.046 ng/ml; IL-1β, 12.40 pg/ml; IL-2, 7.50 pg/ml; IL-4, 8.56 pg/ml; IL-5, 3.10 pg/ml; IL-6, 5.40 pg/ml; IL-8, 20.60 pg/ml; IL-10, 12.90 pg/ml; IL-12p70, 3.40 pg/ml; IL-17, 21.40 pg/ml; TNF-α, 16.50 pg/ml; IFN-α, 8.50 pg/ml; IFN-γ, 23.10 pg/ml.

2.3 | Statistical analysis

Continuous data were expressed as medians (range) when not normally distributed. Differences between groups were tested for significance using the Kruskal-Wallis H test. Statistical analyses

were performed using GraphPad Prism 8. Using MedCalc20.3, receiver operator characteristic (ROC) curves were prepared to determine the sensitivities and specificities of CRP, PCT, and cytokines in comparisons between each of the study groups. The areas under the curve (AUCs) were calculated and the optimal cut-off value was obtained by the largest Youden's Index. Two or more biomarkers were integrated by logistic regression to calculate "comprehensive prediction probabilities," which were then used for the multi-biomarker combination ROC analyses. The *Z* test was used to compare the AUCs. Finally, the sensitivities and specificities of the multi-biomarker combination analyses were calculated by parallel experiments (where any one of multiple criteria was met) and serial experiments (where all the criteria were simultaneously met). A two-sided *p*-value of <0.05 was considered statistically significant.

3 | RESULTS

3.1 | Patient characteristics

A total of 32 children were used as controls (no infection group), and 129 children with infection were subdivided into the following case groups: 33 in the non-sepsis infection group, 67 in the Sepsis 1.0 group, and 29 in the Sepsis 3.0 group (Table 1). The respiratory tract was the most frequent site of infection (Table 1). In the Sepsis 3.0 group, the predominant form of organ dysfunction described was low platelets in 51.72% (15/29), followed by high bilirubin in 31.03% (9/29) of the children. Moreover, 75.86%, (22/29) of the children had a pSOFA score of 2, and 20.69% (6/29) had a pSOFA score of 3. Only 1 child (3.45%) had a pSOFA score of ≥5: PLT2 + CREA3 (Table 1). There were no deaths in our study.

3.2 | Levels of CRP, PCT and cytokine in different groups

Differences in CRP, PCT and cytokine levels are shown in Table 2 and Figure 1. Compared with the no infection group, levels of IL-1β, and IL-8 were significantly increased in the non-sepsis infection group (IL-1β, $p < 0.001$, Figure 1C; IL-8, $p = 0.015$, Figure 1E). In the Sepsis 1.0 group, the levels of CRP (67.08 [1.00 ~ 175.40] mg/L), PCT (0.47 [0.03 ~ 14.26] ng/ml), and IL-6 (81.80 [4.14 ~ 794.86] pg/ml) were significantly higher than those in the non-sepsis infection group (CRP, $p < 0.001$, Figure 1A; PCT, $p < 0.001$, Figure 1B; IL-6, $p < 0.001$, Figure 1D). In the Sepsis 3.0 group, the levels of PCT (2.72 [0.40 ~ 100.00] ng/ml), IL-8 (187.38 [26.51 ~ 7483.49] pg/ml), and IL-10 (50.17 [3.44 ~ 4296.35] pg/ml) were significantly higher than those in the Sepsis 1.0 group (PCT, $p = 0.010$, Figure 1B; IL-8, $p = 0.004$, Figure 1E; IL-10, $p < 0.001$, Figure 1F). No significant differences were found in CRP and IL-6 levels between the Sepsis 1.0 and Sepsis 3.0 groups (Figure 1A,D).

In summary, CRP, PCT, and IL-6 levels showed a significant increase in the hyperinflammatory state, and the levels of PCT, IL-8, and IL-10 were higher in organ dysfunction resulting from dysregulated inflammation. There were no significant increases in the levels of the other cytokines when diagnosed the children with the hyperinflammatory state and those with organ dysfunction (Table S3 and Figure S1).

3.3 | Receiver operating characteristic (ROC) curve analysis

We evaluated the potential of CRP, PCT and IL-6 to distinguish children in the hyperinflammatory state from those with non-sepsis infection (Table 3, Figure 2A,B). The AUCs were 0.974 (CRP), 0.913 (PCT) and 0.919 (IL-6). When cut-offs were set at 25.11 mg/L (CRP), 0.133 ng/ml (PCT), and 35.41 pg/ml (IL-6), the sensitivity rates were 86.57% (CRP), 77.61% (PCT), and 82.09% (IL-6), with specificity rates of 96.97% (CRP), 93.94% (PCT), and 90.91% (IL-6). The following results were obtained when the biomarkers were combined: CRP + PCT (AUC = 0.975); CRP + IL-6 (AUC = 0.978); PCT + IL-6 (AUC = 0.957); CPR + PCT + IL-6 (AUC = 0.978). The sensitivity of diagnosis of the hyperinflammatory state was ≥92.54% if any one of the following three criteria was met: CRP > 25.11 mg/L, PCT > 0.133 ng/ml or IL-6 > 35.41 pg/ml. When two or more of the criteria above were simultaneously met, the specificity of the diagnosis of the hyperinflammatory state reached 100.00%. There were no statistical differences between the AUCs of CRP and any of the above combinations. There were also no statistical differences in the AUCs of the combination of the three biomarkers and any two biomarkers (Table S4).

We further evaluated the ability of PCT, IL-8 and IL-10 to differentiate between children with organ dysfunction caused by dysregulated inflammation from those in the hyperinflammatory state (Table 3, Figure 2C,D). The AUCs were 0.807 (PCT), 0.711 (IL-8), and 0.860 (IL-10). When cut-offs were set at 0.59 ng/ml (PCT), 57.91 pg/ml (IL-8), and 18.40 pg/ml (IL-10), the sensitivity rates were 93.10% (PCT), 86.21% (IL-8), and 72.41% (IL-10), and specificity rates were 58.21% (PCT), 52.24% (IL-8), and 85.07% (IL-10). Combinations of these biomarkers resulted in the following: PCT + IL-8 (AUC = 0.799); PCT + IL-10 (AUC = 0.853); IL-8 + IL-10 (AUC = 0.863); PCT + IL-8 + IL-10 (AUC = 0.860). The sensitivity of diagnosis of organ dysfunction was ≥89.66% if any one of the following three criteria was met: PCT > 0.59 ng/ml, IL-8 > 57.91 pg/ml or IL-10 > 18.40 pg/ml. When all the criteria above were simultaneously met, the specificity of diagnosis of organ dysfunction reached 94.03%. There were no statistical differences between the AUCs of PCT, IL-10 and the any of the above combinations. There were also no statistical differences between the AUCs of a combination of the three biomarkers and a combination of any two biomarkers (Table S5).

Taken together, our results show that CRP, PCT, and IL-6 can be used to diagnose the hyperinflammatory state and that PCT, IL-8,

TABLE 1 Demographic and clinical information of the children included in the study

		No infection	Non-sepsis infection	Sepsis 1.0	Sepsis 3.0
Age in years (range)		4.5 (0.2–13.0)	3.3 (0.2–12.6)	2.7 (0.3–8.8)	2.6 (0.1–16.8)
Gender (Male/Female)		19/13	17/16	38/29	21/8
Peak temperature during admission, °C (range)		/	37.4 (36.9–38.3)	39.4 (37.2–41.5)	39.5 (37.2–40.7)
Hospital stay in days (range)		/	4 (2–9)	5 (3–14)	8 (3–32)
Main source of infection	Respiratory tract	/	28	37	13
	Gastrointestinal tract		1	4	2
	Urogenital tract		0	1	2
	Skin and soft tissue		0	1	2
	Central nervous system		0	0	2
	Respiratory tract + lymphonodus		0	6	1
	Respiratory tract + gastrointestinal tract		3	3	0
	Respiratory tract + skin and soft tissue		0	6	3
	Respiratory tract + urogenital tract		1	4	0
	Respiratory tract + oral cavity		0	1	0
	Gastrointestinal tract + skin and soft tissue		0	1	0
	Gastrointestinal tract + urogenital tract		0	0	1
	Respiratory tract + gastrointestinal tract + skin and soft tissue		0	1	1
	Unknown		0	2	2
Details of pSOFA		/	/	/	PLT2 (12) TBIL2 (9) PLT1 + CREA1 (1) CREA2 + MAP1 (1) PLT3 (3) TBIL2 + MAP1 (1) PLT2 + MAP1 (1) PLT2 + CREA3 (1)

Abbreviations: CREA, serum creatinine; MAP, mean arterial pressure; PLT, platelet count; pSOFA, Pediatric Sequential Organ Failure Assessment; TBIL, serum total bilirubin.

and IL-10 can diagnose organ dysfunction resulting from dysregulated inflammation.

4 | DISCUSSION

To better encompass the pathophysiological processes in sepsis, from the hyperinflammatory state to dysregulated inflammation, we used both SIRS and pSOFA to describe sepsis. In 2015, Kaukonen et al. reported that approximately 12.1% (13,278/109,663) of patients with infection and organ dysfunction did not meet the criteria for SIRS.[16] We speculate that while the hyperinflammatory state was present as an important pathophysiological basis of sepsis, it was not always reflected by the criteria of SIRS. At present, biomarkers are particularly crucial to assist in the assessment of the hyperinflammatory state. In our study, CRP, PCT, and IL-6 levels showed a significant increase in the hyperinflammatory state. Analysis of the ROC showed that CRP, PCT and IL-6 could be used to diagnose the hyperinflammatory state, as all these biomarkers had AUCs of ≥0.9. CRP is a widely used biomarker of infection, although levels rise slowly (6–12 h) after infection and take a long time to peak (1–2 days).[15] Moreover, CRP has only a limited role in diagnosing/predicting severe infections (such as septic shock or bloodstream infection).[15] By contrast, PCT rises rapidly (2–4 h) following infection, peaks quickly (6 h), and shows a significant increase during severe infections.[15] Elevated levels of IL-6 can be detected 1–2 h after infection, peaking within 3 h. IL-6 also has a very short half-life (100 min).[15] Many studies have reported that, in patients with sepsis, persistent IL-6 release is indicative of poor prognosis.[14,15] It is worth noting that the levels of the abovementioned biomarkers might also be increased in non-infectious diseases.[15] Therefore, a comprehensive history, active observation of clinical manifestations, and the rational use of biomarkers are all necessary to diagnose the hyperinflammatory state in pediatric sepsis. In this study, we found that neither PCT nor IL-6

TABLE 2 Median (range) levels of CRP, PCT, and cytokines (IL-1β, IL-6, IL-8 and IL-10) in the study groups

	CRP (mg/L)	PCT (ng/ml)	IL-1β (pg/ml)	IL-6 (pg/ml)	IL-8 (pg/ml)	IL-10 (pg/ml)
no infection	1.00 (1.00–4.19)	0.03 (0.02–0.12)	0.00 (0.00–16.37)	2.18 (0.46–17.53)	20.33 (2.72–46.33)	1.645 (0.61–7.00)
non-sepsis infection	1.00 (1.00–33.3)	0.06 (0.02–0.84)	13.47 (0.00–123.57)	11.81 (0.00–170.25)	49.86 (4.56–548.57)	3.52 (1.04–114.19)
Sepsis 1.0	67.08 (1.00–175.40)	0.47 (0.03–14.26)	15.13 (0.00–114.87)	81.80 (4.14–794.86)	57.46 (7.01–2472.56)	4.82 (1.70–205.77)
Sepsis 3.0	71.01 (2.15–197.03)	2.72 (0.40–100.00)	10.99 (0.00–130.98)	209.06 (13.02–7585.29)	187.38 (26.51–7483.49)	50.17 (3.44–4296.35)

Abbreviations: CRP, C-reactive protein; IL, interleukin; PCT, procalcitonin.

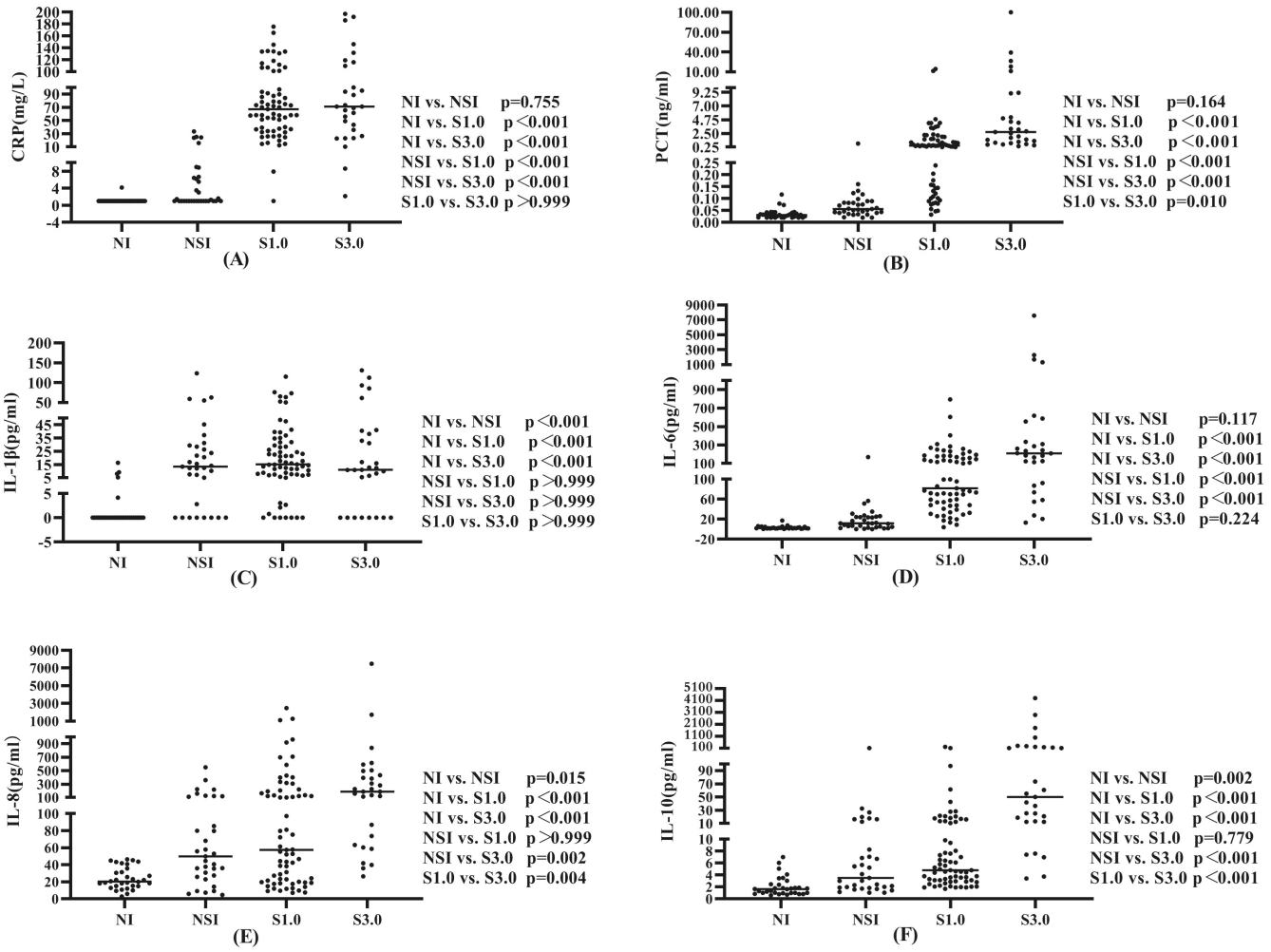

FIGURE 1 Serum CRP, PCT, and cytokine (IL-1β, IL-6, IL-8, IL-10) levels in the study groups. (A) CRP, (B) PCT, (C) IL-1β, (D) IL-6, (E) IL-8, (F) IL-10. NI, no infection, NSI, non-sepsis infection; S1.0, Sepsis 1.0; S3.0, Sepsis 3.0; CRP, C-reactive protein; PCT, procalcitonin; IL, interleukin

were better able to diagnose the hyperinflammatory state than CRP. We believe that this is because there was ample time from infection to the hospital visit for CRP levels to rise sufficiently. Moreover, the hyperinflammatory state itself is not severe, masking obvious changes in PCT and IL-6. We found that, in a combination of any two of CRP, PCT and IL-6 (i.e., PCT with CRP, CRP with IL-6, and PCT with IL-6), when any one of the two biomarkers met the criteria of CRP >25.11 mg/L, PCT 0.133 ng/ml or IL-6 > 35.41 pg/ml, the diagnostic sensitivity was ≥92.54%. Moreover, when both biomarkers were simultaneously elevated, the diagnostic specificity reached 100.00%. Therefore, we believe that the combination of any two of CRP, PCT and IL-6 should be used to better diagnose the hyperinflammatory state.

In our study, PCT, IL-8, and IL-10 levels showed a significant increase in organ dysfunction. Analysis of the ROC showed that PCT, IL-8, and IL-10 were good indicators of organ dysfunction resulting

TABLE 3 The application of the ROC curve in Sepsis 1.0 and Sepsis 3.0

	AUC	Cut-off	Sensitivity	95% CI	Specificity	95% CI	Youden Index
Sepsis 1.0							
CRP	0.974	>25.11 g/L	86.57%	76.0%–93.7%	96.97%	84.2%–99.9%	0.84
PCT	0.913	>0.133 ng/ml	77.61%	65.8%–86.9%	93.94%	79.8%–99.3%	0.72
IL-6	0.919	>35.41 pg/ml	82.09%	70.8%–90.4%	90.91%	75.7%–98.1%	0.73
CRP + PCT	0.975	CRP > 25.11 g/L or PCT > 0.133 ng/ml	92.54%		90.91%		
		CRP > 25.11 g/L and PCT > 0.133 ng/ml	71.64%		100.00%		
CRP + IL-6	0.978	CRP > 25.11 g/L or IL-6 > 35.41 pg/ml	92.54%		87.88%		
		CRP > 25.11 g/L and IL-6 > 35.41 pg/ml	76.12%		100.00%		
PCT + IL-6	0.957	PCT > 0.133 ng/ml or IL-6 > 35.41 pg/ml	94.03%		84.85%		
		PCT > 0.133 ng/ml and IL-6 > 35.41 pg/ml	65.67%		100.00%		
CRP + PCT + IL-6	0.978	CRP > 25.11 g/L or PCT > 0.133 ng/ml or IL-6 > 35.41 pg/ml	95.52%		81.82%		
		CRP > 25.11 g/L and PCT > 0.133 ng/ml and IL-6 > 35.41 pg/ml	62.69%		100.00%		
Sepsis 3.0							
PCT	0.807	>0.59 ng/ml	93.10%	77.2%–99.2%	58.21%	45.5% -70.2%	0.51
IL-8	0.711	>57.91 pg/ml	86.21%	68.3%–96.1%	52.24%	39.7%–64.6%	0.38
IL-10	0.860	>18.40 pg/ml	72.41%	52.8%–87.3%	85.07%	74.3%–92.6%	0.57
PCT + IL-8	0.799	PCT > 0.59 ng/ml or IL-8 > 57.91 pg/ml	96.55%		34.33%		
		PCT > 0.59 ng/ml and IL-8 > 57.91 pg/ml	82.76%		76.12%		
PCT + IL-10	0.853	PCT > 0.59 ng/ml or IL-10 > 18.40 pg/ml	93.10%		49.25%		
		PCT > 0.59 ng/ml and IL-10 > 18.40 pg/ml	72.41%		94.03%		
IL-8 + IL-10	0.863	IL-8 > 57.91 pg/ml or IL-10 > 18.40 pg/ml	89.66%		49.25%		
		IL-8 > 57.91 pg/ml and IL-10 > 18.40 pg/ml	68.97%		88.06%		
PCT + IL-8 + IL-10	0.860	PCT > 0.59 ng/ml or IL-8 > 57.91 pg/ml or IL-10 > 18.40 pg/ml	96.55%		31.34%		
		PCT > 0.59 ng/ml and IL-8 > 57.91 pg/ml and IL-10 > 18.40 pg/ml	68.97%		94.03%		

Abbreviations: CRP, C-reactive protein; IL, interleukin; ROC, receiver operating characteristic; PCT, procalcitonin.

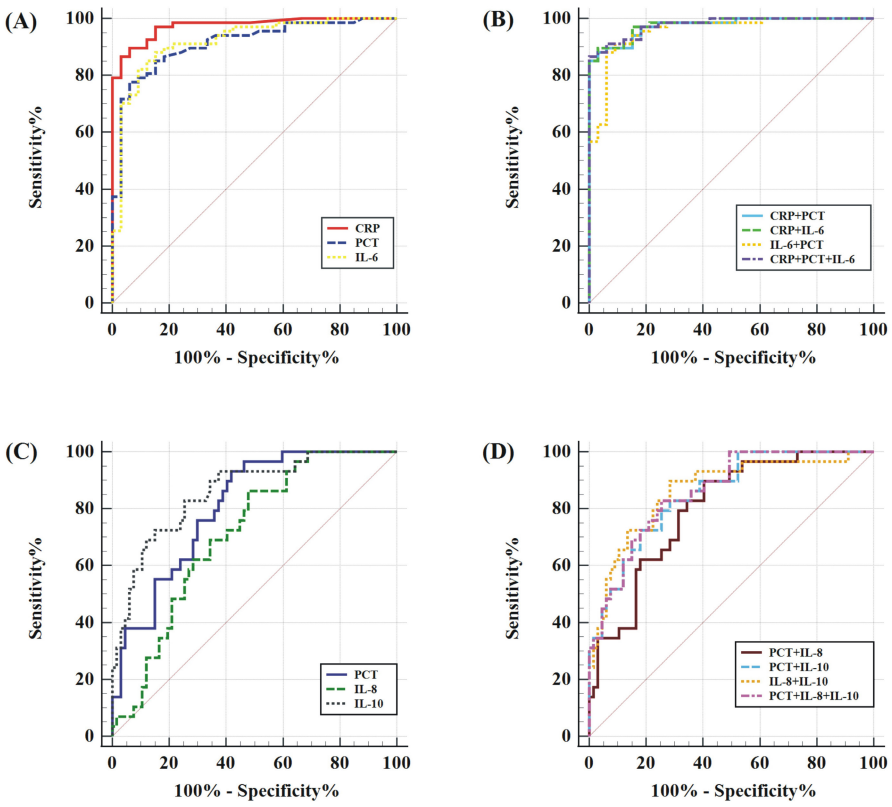

FIGURE 2 ROC analyses of key biomarker levels in the study groups. (A) ROC analysis of CRP, PCT and IL-6 distinguishes children in the hyperinflammatory state from those with non-sepsis infections; (B) ROC analysis of combinations of CRP, PCT and IL-6 distinguishes children in the hyperinflammatory state from those with non-sepsis infections; (C) ROC analysis of PCT, IL-8 and IL-10 distinguishes children with dysregulated inflammation from those in the hyperinflammatory state; (D) ROC analysis of combinations of PCT, IL-8 and IL-10 distinguishes children with dysregulated inflammation from those in the hyperinflammatory state. ROC, receiver operating characteristic; CRP, C-reactive protein; PCT, procalcitonin; IL, interleukin

from dysregulated inflammation, with AUCs of 0.807 (PCT), 0.711 (IL-8), and 0.860 (IL-10). All three biomarkers have previously been reported to play a role in sepsis. After severe infection, PCT increases rapidly, and this has been used as an indicator in the diagnosis of pneumonia, bloodstream infections, severe sepsis, and septic shock. High levels of PCT in the acute phase of infection has been associated with poor prognosis.[17–20] As a neutrophil chemotactic factor, IL-8 is an important pro-inflammatory cytokine that stimulates systematic inflammation. Studies have reported that early levels of IL-8 show a strong positive correlation with the stage of sepsis, the SOFA scores, and mortality within 28 days.[21,22] Compared with other biomarkers (such as IL-6), IL-8 is better associated with sepsis severity and mortality within the first day.[23] In addition, IL-8 (above 234 pg/ml) had a sensitive predictive value for sepsis and multiple organ dysfunction syndrome (MODS) in children with hyperinflammation (such as after burns).[24] On the other hand, IL-10, an important anti-inflammatory cytokine, plays a key role in CARS. Persistent over-production of IL-10 leading to immunosuppression is the main risk factor for sepsis severity and fatal outcome.[5] In patients with sepsis, high levels of IL-10 are associated with septic shock, MODS and even death.[14,19,25] Apart from over-production, the abnormal early release of IL-10 also causes immunosuppression that results in dysregulated inflammation.[26] Our study found that the AUC of IL-10 was a better diagnostic biomarker than IL-8 ($p = 0.006$). We, therefore, hypothesize that, compared with the continued release of the pro-inflammatory cytokines, the immunosuppression caused by the premature and excessive release of anti-inflammatory cytokines plays a more critical role in progression from the hyperinflammatory state to dysregulated inflammation. Apart from IL-8, the AUCs of each of the combinations of biomarkers were not significantly different from those of PCT or IL-10. However, a combination of three biomarkers, when any one of the three biomarkers met the diagnostic criteria (PCT > 0.59 ng/ml, IL-8 > 57.91 pg/ml, IL-10 > 18.40 pg/ml) resulted in a sensitivity of identifying dysregulated inflammation of 96.55%. Moreover, when simultaneous elevation of these biomarkers occurred, the diagnostic specificity was 94.03%. Therefore, to enhance the sensitivity and specificity of diagnosis and minimize poor prognosis, we believe that it is necessary to use a combination of all three biomarkers.

In our study, IL-6 failed to distinguish children with dysregulated inflammation from those in the hyperinflammatory state. However, multiple studies have suggested that IL-6 plays a key role in the progression of sepsis. In those studies, the difference in disease severity between the severe infection and non-severe infection groups was considerable (such as the presence or absence of septic shock and differences in survival status).[14,19,21,22] In our study, the distinction between the Sepsis 1.0 and Sepsis 3.0 groups was not as considerable as that between the severe infection and non-severe infection groups mentioned above; therefore, differences in IL-6 levels might not be easily detectable. Moreover, some studies have proposed that IL-6 is less sensitive for early detection of special consequences such as organ dysfunction in the hyperinflammatory state.[24] Therefore, early levels of IL-6 may not be good predictors of sepsis progression. Sequential measurement of IL-6 during treatment has been shown to be more valuable in evaluating disease severity and in predicting the outcome of

sepsis.[21,27] In our study, the lack of significant differences in IL-6 levels between the Sepsis 1.0 and Sepsis 3.0 groups might also be attributed to the relatively small number of cases in the dysregulated inflammation group.

In conclusion, our study showed that a combination of any two of IL-6, CRP and PCT is excellent for early diagnosis of the hyperinflammatory state. We also showed that PCT, IL-8 and IL-10 all play a role in organ dysfunction induced by dysregulated inflammation, and that a combination of all three biomarkers can be used as a good diagnostic tool for organ dysfunction. Therefore, monitoring these biomarkers is essential for the early diagnosis of pediatric sepsis and to improve prognosis in children.

AUTHOR CONTRIBUTIONS

ZGB and CD conceptualized and designed the study, coordinated and supervised data collection, performed the preliminary data analysis, and drafted the initial manuscript. ZRX, ZXF, YCY, and THT collected the data, performed the laboratory data analysis, supervised the laboratory techniques, and performed the outcome assessments. WYD and SWB conceptualized and designed the study, and critically reviewed and revised the manuscript. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

ACKNOWLEDGMENT

This study was supported by grants from The Science and Technology Plan Guidance Project of Hangzhou (Nos, 20191231Y049, 20201231Y051) and The Municipal Key Discipline of Hangzhou of Respiratory Diseases and Infectious Diseases in Children.

CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors.

DATA AVAILABILITY STATEMENT

The data used to support the findings of this study are available from the corresponding author upon request.

ORCID

Yidong Wu https://orcid.org/0000-0003-0803-657X

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Zeng G, Chen D, Zhou R, et al. Combination of C-reactive protein, procalcitonin, IL-6, IL-8, and IL-10 for early diagnosis of hyperinflammatory state and organ dysfunction in pediatric sepsis. *J Clin Lab Anal*. 2022;36:e24505. doi: 10.1002/jcla.24505