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Role of hepcidin in the diagnosis of sepsis and septic shock in children

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Background/aim: The purpose of this study is to compare the diagnostic value of hepcidin level with the white blood cell (WBC), C-reactive protein (CRP), procalcitonin (PCT), and interleukin-6 (IL-6) levels in pediatric sepsis and septic shock.

Materials and methods: A cohort of 89 individuals were divided into four groups: a healthy control group (HCG, n = 28), pediatric intensive care unit control group (PICUCG, n = 17), sepsis group (SG, n = 23), and septic shock group (SSG, n = 21). WBC, CRP, PCT, IL-6, and hepcidin levels were studied in the PICUCG, SG, and SSG, while hepcidin and IL-6 levels were studied in the HCG.

Results: In distinguishing the SG and SSG from the HCG, hepcidin sensitivity and specificity were found to be 100%. Distinguishing between the PICUCG and the SG, hepcidin sensitivity was calculated as 95.6% and specificity was calculated as 100%. The sensitivity of WBC, CRP, and PCT was lower than that of hepcidin, but the sensitivity of IL-6 was higher than that of hepcidin. While the specificity of PCT and IL-6 was the same as hepcidin, the specificity of WBC and CRP was lower than that of hepcidin.

Conclusion: Hepcidin is a more reliable indicator than WBC and CRP levels in distinguishing children with sepsis and septic shock from healthy children and nonseptic pediatric ICU patients.

Key words: C-reactive protein, hepcidin, interleukin-6, procalcitonin, sepsis, septic shock

1. Introduction

Sepsis is a systemic, excessive, uncontrolled, and deleterious host response syndrome that is associated with a documented or suspected infection with the systemic inflammatory response syndrome (1). Despite improved understanding of the pathophysiology, advanced intensive care technology, and available pharmacological therapies, sepsis remains the most common cause of morbidity and mortality in infants and children worldwide (2). It can rapidly progress to septic shock, multiorgan failure, and death if treatment is delayed. Therefore, early identification of sepsis and prompt intervention are cornerstones to reduce the morbidity and mortality. For all of these reasons, recent clinical trials focused on new biochemical markers in order to increase early recognition of sepsis (3).

Hepcidin, synthesized in hepatocytes and the master hormonal regulator of iron metabolism, is an antimicrobial peptide and acute-phase reactant. Synthesis of hepcidin is regulated by interleukin-6 (IL-6) and lipopolysaccharide in inflammatory and infectious situations (4,5). It binds to the iron exporter ferroportin and promotes its internalization and lysosomal degradation in target cells, especially macrophages, enterocytes, and hepatocytes. As a result, the excessive hepcidin synthesis causes hypoferremia by inhibiting dietary iron absorption and enhancing intracellular iron sequestration (6). Hepcidin has direct antimicrobial activity and it contributes to host defense by depriving microorganisms of this essential iron mineral (6,7). The use of hepcidin as an adjunct test for sepsis has been detected in adults, as well as in term and preterm neonates (8,9).

The aim of this prospective cross-sectional study is to compare the diagnostic value of hepcidin with well-established biochemical markers such as white blood cell (WBC), C-reactive protein (CRP), procalcitonin (PCT), and IL-6 levels in pediatric sepsis and septic shock.

2. Materials and methods

This prospective cross-sectional study was conducted between June 2015 and July 2016 at the University of

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Health Sciences Bakırköy Dr. Sadi Konuk Research and Training Hospital in Turkey. A total of 89 individuals, 61 pediatric patients hospitalized in a 9-bed pediatric intensive care unit (PICU) and 28 healthy children routinely examined at a pediatric outpatient clinic, were included in the study. The study was initiated following the approval of the Bakırköy Dr. Sadi Konuk Research and Training Hospital Ethics Committee (approval number: 2015/08/06; approval date: 11 May 2015). The individuals were divided into four groups: healthy control group (HCG), PICU control group (PICUCG), sepsis group (SG), and septic shock group (SSG). Pediatric sepsis and septic shock were defined according to international guidelines (1). The PICUCG was composed of nonseptic patients who were followed up in the PICU. Informed consent was obtained from patients' parents or legal guardian. In the PICUCG, SG, and SSG, CRP, WBC, PCT, IL-6, and hepcidin levels were studied in the remaining blood serum sample taken for routine laboratory tests immediately after admission to the PICU. In the healthy children, venous blood samples were collected in tubes from the antecubital vein for routine laboratory tests followed by overnight fasting. Hepcidin and IL-6 were studied in the remaining blood serum sample taken from the healthy children. The tubes were centrifuged at 2000 × *g* (10 min) to remove the serum. The serum samples were kept at –80 °C until analysis of hepcidin and IL-6. Serum levels of hepcidin and IL-6 were assessed by the enzyme-linked immunosorbent assay (ELISA) technique. Serum hepcidin and IL-6 levels were determined using a Human Hepcidin ELISA kit (catalog no: CK-E90190) purchased from Eastbiopharm (China) and a Human IL-6 ELISA kit (catalog no: 950.030.192) purchased from Diaclone (France) following the manufacturer's instructions. Serum levels were expressed as ng/mL. The intra- and interassay coefficients of variation were 7.9% and 4.2% and 8.1% and 7.7%, respectively. Serum PCT and CRP levels were determined by the Roche Cobas 8000 Clinical-Immunochemistry Integrated Automation System using commercial kits (Roche Diagnostics, USA). Hematological tests for WBC count was performed using the Abbott Cell-Dyn Ruby analyzer (Abbott Diagnostics, USA).

All statistical analyses were performed using IBM SPSS for Windows, version 20.0 (IBM Corp., Armonk, NY, USA). Kolmogorov–Smirnov tests were used to test the normality of data distribution. Continuous variables were expressed as mean ± standard deviation and median (25th–75th percentiles), and categorical variables were expressed as counts (percentages). Differences between the groups were analyzed by one-way ANOVA and Tukey post hoc test, Kruskal–Wallis one-way ANOVA, and Dunn post hoc test for numerical variables with normal and abnormal distribution while the Monte Carlo chi-square test was used for categorical variables. The relationship between numerical variables was evaluated by Spearman correlation analysis. Receiver operating curve (ROC) analysis was performed to determine the sensitivity and the specificity of different cutoff points for WBC, CRP, PCT, IL-6, and hepcidin to predict sepsis and septic shock. The most appropriate cutoff point was chosen according to the ROC analysis and the area under the curve (AUC) was calculated. $P < 0.05$ was considered statistically significant.

3. Results

The mean age of participants was 46.7 ± 37.4 months. Of them, 53 (59.6%) were male and 36 (40.4%) were female. There was a significant difference between the groups in terms of the mean age ($P = 0.04$). As a result of the binary comparisons made to determine the difference, the mean age of the HCG was statistically and significantly higher than that of the SG and SSG ($P < 0.05$). There was no significant difference in the mean age between the PICUCG, SG, and SSG ($P = 0.18$). There was no significant difference between the groups in terms of sex ($P = 0.21$). The diagnosis distribution of the pediatric intensive care unit control group is shown in Table 1. Distribution of the causative organisms in the sepsis and septic shock group is shown in Table 2.

There was a significant difference between the groups in terms of the WBC, CRP, PCT, IL-6, and hepcidin levels (respectively $P = 0.029$, $P = 0.001$, $P = 0.003$, $P < 0.001$, and $P < 0.001$) (Table 3). As a result of the binary comparisons made to determine the difference, hepcidin and IL-6 levels were significantly lower in the HCG compared to the PICUCG, SG, and SSG ($P < 0.05$, Table 3). Hepcidin, WBC, CRP, PCT, and IL-6 levels were significantly lower in the PICUCG compared to the SG and SSG ($P < 0.05$, Table 3). There was no significant difference between the SG and SSG in terms of the WBC, CRP, PCT, IL-

Table 1. The diagnoses of the pediatric intensive care unit control group.

Patients' diagnoses (n: 17)	Number (%)
Trauma	6 (35.29)
Pneumonia	3 (17.64)
Hemolytic uremic syndrome	2 (11.76)
Intoxication	1 (5.88)
Intracranial mass	1 (5.88)
Metabolic disease	1 (5.88)
Guillain–Barre syndrome	1 (5.88)
Fulminant autoimmune hepatitis	1 (5.88)
Status epilepticus	1 (5.88)

Table 2. Distribution of causative organisms in the sepsis and septic shock group.

Sepsis (n: 23)	
Methicillin-resistant coagulase negative staphylococci (blood)	3
Acinetobacter baumannii (tracheal aspirate)	2
Methicillin-sensitive coagulase negative staphylococci (CSF)	1
Methicillin-sensitive coagulase negative staphylococci (tracheal aspirate)	1
Pseudomonas aeruginosa (tracheal aspirate)	1
Streptococcus pneumonia (CSF)	1
Neisseria meningitis (serum-PCR)	1
Cytomegalovirus (serum-PCR)	1
Influenza A virus (nasopharyngeal aspirate-PCR)	1
Total	12 (52.17%)
Septic shock (n: 21)	
Pseudomonas aeruginosa (tracheal aspirate)	1
Pseudomonas aeruginosa (blood)	1
Klebsiella pneumoniae (tracheal aspirate)	1
Neisseria meningitis (serum-PCR)	1
Leptospirosis (positivity of serum IgG and IgM))	1
Total	5 (23.8%)

CSF: Cerebrospinal fluid, PCR: polymerase chain reaction.

6, and hepcidin levels ($P > 0.05$, Table 3). The point distribution of the hepcidin levels in the groups is shown in Figure 1. A statistically significant correlation was observed between hepcidin and Pediatric Risk of Mortality (PRISM) III score ($r = 0.588$, $P < 0.001$) and also between hepcidin and IL-6 ($r = 0.757$, $P < 0.001$) (Figure 2). Correlation of the biomarkers between each other and the correlation between biomarkers and the PRISM III score are shown in Table 4.

According to the ROC analysis, when the cutoff value for hepcidin was determined to be 18.8 ng/mL according to the healthy children, its sensitivity and specificity for a diagnosis of sepsis and septic shock were found to be 100% (Figure 3). When the cutoff value for IL-6 was determined to be 0.02 ng/mL according to the healthy children, its sensitivity and specificity for a diagnosis of sepsis and septic shock were the same as that reported for hepcidin (100%). The cutoff value for hepcidin was determined to be 54.3 ng/mL in distinguishing between the PICUCG and the SG. The sensitivity and specificity for this value were calculated as 95.6% and 100%, respectively (Table 5; Figure 3). In distinguishing between these two groups, the sensitivity of WBC, CRP, and PCT levels was lower than that of hepcidin (respectively 78.2%, 78.2%, and 60.8%), but the sensitivity of IL-6 was higher than that of hepcidin (100%). While the specificity of PCT and IL-6 was the same as that of hepcidin (100%), the specificity of WBC and CRP levels was lower than that of hepcidin (respectively 58.8% and 82.3%) (Table 5). The cutoff value for hepcidin level was determined to be 54.3 ng/mL in distinguishing between the PICUCG and the SSG. The sensitivity and specificity for this value were calculated as 100% and 100%, respectively (positive predictive value = 100%, negative predictive value = 100%) (Figure 3). In distinguishing between these two groups, the sensitivity and specificity of IL-6 were the same as that of hepcidin. The sensitivity and specificity of WBC count and CRP were lower than that of hepcidin (respectively, sensitivity/specificity: 71.4%/70.6% and 76.2%/82.3%). While the sensitivity of PCT was lower than that of hepcidin (71.4%), the specificity of PCT was the same as that of hepcidin (100%).

4. Discussion

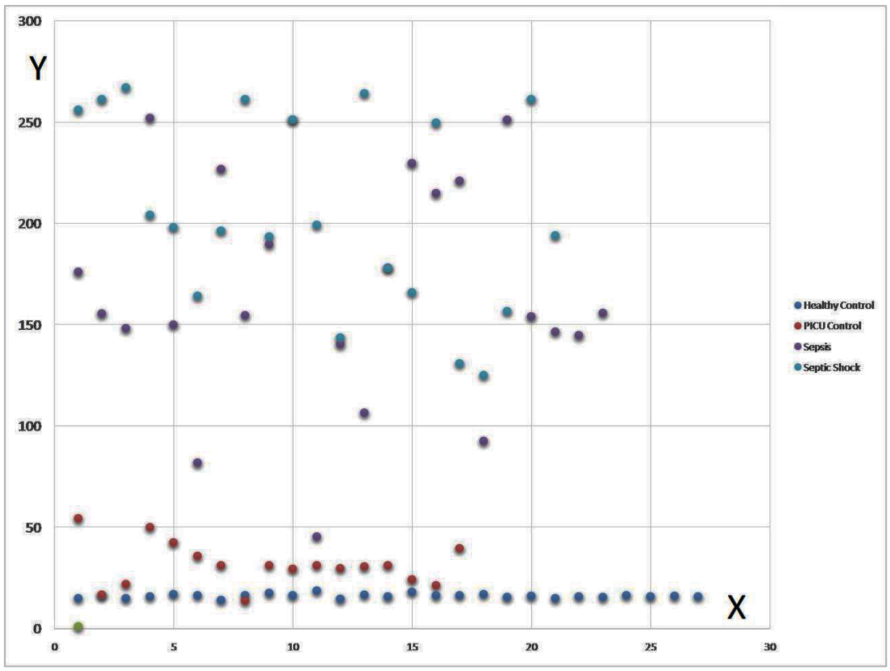
In humans, high levels of hepcidin were detected in patients with inflammatory diseases and chronic infections. Among the cytokines, IL-6, but not tumor necrosis factor α or IL-1α, strongly stimulates hepcidin mRNA synthesis. Sepsis and septic shock are prototypical acute and excessive inflammatory situations. In recent studies, the hepcidin level was higher in adult septic patients compared to healthy individuals and nonseptic ICU patients (6,8,10–12). As a result of our study, hepcidin levels were found to be significantly higher in the patients with sepsis and septic shock compared to both the healthy children and the nonseptic PICU patients. While the sensitivity and specificity of hepcidin were 100% in distinguishing the children with sepsis from the healthy children, its sensitivity and specificity were respectively

Table 3. The characteristics of the study population groups.

Patients' characteristics and serum markers	[1]Healthy controls (n: 28)	[2]Pediatric intensive care unit control (n: 17)	[3]Sepsis (n: 23)	[4]Septic shock (n: 21)	P	P[d]
Age (months), mean ± SD	58.78 ± 28.78	56.35 ± 35.55	34.52 ± 40.97	36.24 ± 40.34	0.04[a]	
Male sex, n (%)	17 (60.7%)	13 (76.5%)	14 (60.9%)	9 (42.9%)	0.338[b]	
PRISM III, median (25th-75th percentiles)		0 (0–8)	12 (8–17)	25 (16–29)	<0.001	P^{2-3}: 0.01 P^{2-4}: <0.001 P^{3-4}: 0.002
WBC count (/mm^3)		9.7 (6.85–12.7)	16.6 (10.6–29.6)	13.6 (7.8–21.5)	0.029[c]	P^{2-3}: 0.01 P^{2-4}: 0.047 P^{3-4}: 0.55
CRP, mg/dL		0.2 (0.065–0.3)	2.1 (0.4–5)	3.4 (0.35–11.3)	0.001[c]	P^{2-3}: 0.002 P^{2-4}: <0.001 P^{3-4}: 0.53
PCT, ng/dL		0.2 (0.075–0.45)	1.6 (0.1–9.2)	8.1 (0.15–60)	0.003[c]	P^{2-3}: 0.014 P^{2-4}: 0.001 P^{3-4}: 0.31
IL-6, ng/mL	0.013 (0.010–0.017)	0.026 (0.021–0.057)	0.167 (0.127–0.188)	0.177 (0.130–0.225)	<0.001[c]	P^{1-2}: 0.013 P^{1-3} <0.001 P^{1-4}: <0.001 P^{2-3}: <0.001 P^{2-4}: <0.001 P^{3-4}: 0.67
Hepcidin, ng/mL	16 (15.6–16.5)	31.2 (23–37.7)	155.5 (144.7–221.1)	198.2 (165.1–258.6)	<0.001[c]	P^{1-2}: 0.015 P^{1-3}: <0.001 P^{1-4}: <0.001 P^{2-3}: 0.001 P^{2-4}: <0.001 P^{3-4}: 0.24

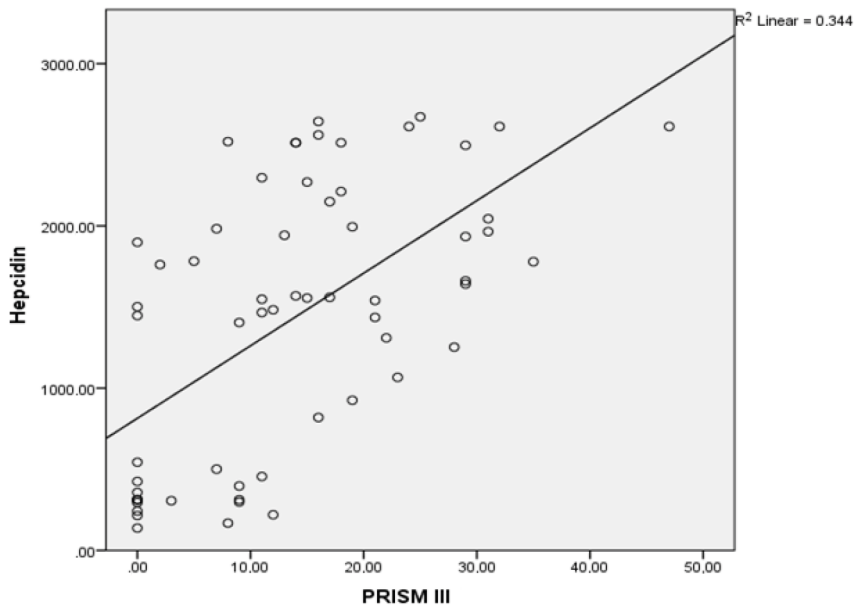
[a] One-way ANOVA, [b] chi-square test, [c] Kruskal–Wallis test, [d] Dunn post hoc test.
Data are expressed as mean ± SD for age and as median (IQR) for WBC, CRP, PCT, IL-6, and hepcidin levels.
WBC: White blood cell, CRP: C-reactive protein, PCT: procalcitonin, IL-6: interleukin-6

95.6% and 100% in distinguishing the children with sepsis from the nonseptic PICU patients (Table 5; Figure 3). In distinguishing the children with sepsis from the nonseptic PICU patients, while only the sensitivity of IL-6 was higher than that of hepcidin, the specificity of PCT and IL-6 was the same as that of hepcidin (100%). The sensitivity and specificity of WBC and CRP levels for a diagnosis of sepsis were found to be lower than that of hepcidin (Table 5). In our study, there was no significant difference between the SG and SSG in terms of the values of hepcidin and all other markers (Table 3). Studies showing the relationship between hepcidin and sepsis in children were performed in the neonatal patient population (9,13). Cizmeci et al. (9) showed that the range of cord blood hepcidin was found to be significantly increased in 38 newborns with early-onset neonatal sepsis compared to healthy controls. Unlike our study, they did not compare hepcidin with other sepsis markers. Wu et al. (13) observed that hepcidin level was four times greater in 17 newborns with late-onset neonatal sepsis compared to healthy controls. Similarly, in this study, the sensitivity and specificity of hepcidin for a diagnosis of sepsis were found to be higher than that of CRP. To the best of our knowledge, our study is the first study to investigate the relationship between hepcidin and sepsis in children except for the newborn period. In addition, the comparison of WBC, CRP, PCT, IL-6, and hepcidin values in a diagnosis of sepsis and septic shock is very important.



PICU: Pediatric intensive care unit.

Figure 1. The distribution of hepcidin levels (ng/mL) in the groups.



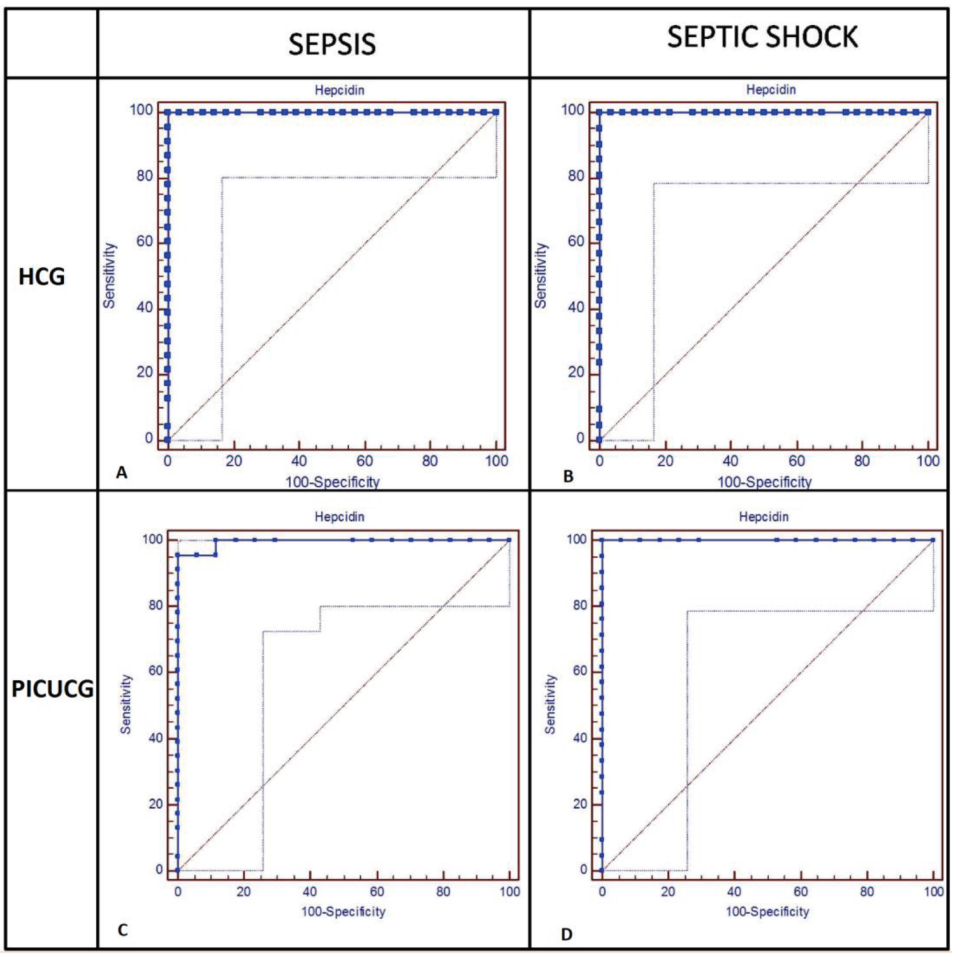
PRISM III: Pediatric Risk of Mortality

Figure 2. Correlation between hepcidin and PRISM III score. PRISM III: Pediatric Risk of Mortality III.

Table 4. Correlation of the biomarkers between each other and correlation between biomarkers and Pediatric Risk of Mortality III score.

Parameters		WBC (/mm³)	CRP (mg/dL)	PCT (ng/dL)	IL-6 (ng/mL)	Hepcidin (ng/mL)	PRISM III
WBC (/mm³)	r P		0.108 0.407	0.013 0.919	0.372 0.003	0.217 0.093	–0.048 0.711
CRP (mg/dL)	r P	0.108 0.407		0.536 <0.001	0.321 0.012	0.379 0.003	0.415 0.001
PCT (ng/dL)	r P	0.013 0.919	0.536 <0.001		0.308 0.016	0.338 0.008	0.480 <0.001
IL-6 (ng/mL)	r P	0.372 0.003	0.321 0.012	0.308 0.016		0.757 <0.001	0.582 <0.001
Hepcidin (ng/mL)	r P	0.217 0.093	0.379 0.003	0.338 0.008	0.757 <0.001		0.588 <0.001
PRISM III	r P	–0.048 0.711	0.415 0.001	0.480 <0.001	0.582 <0.001	0.588 <0.001	

WBC: White blood cell, CRP: C-reactive protein, PCT: procalcitonin, IL-6: interleukin-6, PRISM III: Pediatric Risk of Mortality III.



HCG: Healthy control group**, PICUCG:** Pediatric intensive care unit control group

Figure 3. The diagnosis of sepsis and septic shock for hepcidin according to receiver operating curve (ROC) analysis. A) Sepsis group versus healthy control group [area under the curve (AUC) = 1.000], B) septic shock group versus healthy control group (AUC = 1.000), C) sepsis group versus pediatric intensive care unit control group (AUC =0.995), D) septic shock group versus pediatric intensive care unit control group (AUC = 1.000).

Table 5. According to receiver operating curve analysis, the cutoff, sensitivity, specificity, and positive and negative predictive values of hepcidin and other serum markers in distinguishing between the pediatric intensive care unit control group and the sepsis group.

	Diagnostic scan					ROC curve		
Serum markers	Cutoff	Sens	Spec	PPV	NPV	AUC	95% CI	P
WBC (/mm^3)	10.2	78.26	58.82	72.0	66.7	0.738	0.575–0.864	<0.01
CRP (mg/dL)	0.3	78.26	82.35	85.7	73.7	0.812	0.657–0.918	<0.01
PCT (ng/dL)	1.27	60.87	100	100	65.4	0.752	0.590–0.875	<0.01
IL-6 (ng/mL)	0.712	100	100	100	100	1.000	0.912–1.000	<0.01
Hepcidin (ng/mL)	54.3	95.65	100	100	94.4	0.995	0.902–1.000	<0.01

WBC: White blood cell, CRP: C-reactive protein, PCT: procalcitonin, IL-6: interleukin-6., Sens: sensitivity, Spec: specificity, PPV: positive predictive value, NPV: negative predictive value, AUC: area under the curve, CI: confidence interval.

In distinguishing between sepsis and noninfective causes of inflammation in children and adults, the results of our study are consistent with the literature in that the specificity of PCT was higher than that of CRP, but they differ from the literature in that the sensitivity of CRP was higher than that of PCT (14–18). It was found in many child and adult studies that sepsis severity was associated with PCT and IL-6 but not associated with CRP (15,17,19–24). The results of our study were consistent with the literature in terms of CRP, but differ from the literature in that there was no significant difference between PCT and IL-6 in distinguishing between sepsis and septic shock.

The limitations of our study are primarily that the number of patients was low and the mean age of the healthy children was significantly higher than that of other groups. Moreover, we were not able to examine WBC, CRP, and PCT levels due to inadequate blood volume collected from the healthy children. The levels of the biomarkers at the time the samples were taken may not reflect their actual levels because our data are cross-sectional, the half-lives of sepsis markers are different (14,25–27), and the majority of patients had received antibiotic and fluid therapy prior to the PICU. Hepcidin is severely affected by anemia parameters such as serum and storage iron levels of patients regardless of the level of inflammation. Body weight and body mass index are very important factors affecting hepcidin and the levels of other biochemical markers (28,29). These factors were not compared between the groups in our study. In addition, various factors such as acute kidney injury, liver injury, nutritional status, severity of illness, treatment approaches, and other comorbidities, which are likely to influence hepcidin and other biomarker levels, were not evaluated in our study.

In conclusion, our study has showed that hepcidin is a more reliable indicator than WBC count and CRP in distinguishing children with sepsis and septic shock from healthy children and nonseptic PICU patients. However, it is not reliable in distinguishing between sepsis and septic shock in children. Although our study does not provide any new pathway of the sepsis pathophysiology or any new change to our clinical management, we think that there is a need for more comprehensive studies on this subject.

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