Diagnostic markers of serious bacterial infections in infants aged 29 to 90 days

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ABSTRACT

Objectives: The diagnosis of serious bacterial infection (SBI) is difficult due to a lack of clinical evidence. The purpose of this study was to determine which inflammatory markers can be used to detect SBI in febrile infants.

Methods: This retrospective cohort study included infants aged 29 to 90 days who visited a tertiary hospital emergency department in Korea between July 2016 and June 2018. The diagnostic characteristics of the neutrophil-to-lymphocyte ratio (NLR), procalcitonin (PCT), C-reactive protein (CRP), white blood cell (WBC) count, and absolute neutrophil cell (ANC) count for detecting SBI were described. Their cutoff values were calculated based on receiver operating characteristic (ROC) curve analysis.

Results: Among 528 infants, 199 were finally enrolled. SBI was detected in 68 (34.2%) of these infants. The median values of all investigated diagnostic markers were significantly higher in infants with SBI than the values in those without: WBC (12.72 vs. 9.91 k/μL), ANC (6.28 vs. 3.14 k/μL), CRP (26.6 vs. 2.8 mg/L), NLR (1.29 vs. 0.78), and PCT (0.5 vs. 0 ng/mL). The areas under the ROC curves for discriminating SBI were: 0.705 (95% confidence interval [CI], 0.629-0.781), 0.793 (95% CI, 0.731-0.856), 0.832 (95% CI, 0.775-0.889), 0.722 (95% CI, 0.651-0.792), and 0.695 (95% CI, 0.611-0.780) for WBC, ANC, CRP, NLR, and PCT, respectively. Using a cutoff value of 0.67 for NLR, the negative predictive value was 90.8% for identifying SBI.

Conclusions: CRP was the best single discriminatory marker of SBI, while NLR was the best parameter for considering discharge.

Key words: bacterial infection; urinary tract infection; clinical marker; discharge planning

INTRODUCTION

Fever is the most common chief complaint of pediatric patients visiting the emergency department. It also accounts for 30 percent of pediatric patients who visit outpatient clinics. However, 20 percent of these febrile patients have no definite fever focus on history taking and physical examination (1). Although diagnosis is difficult due to a lack of clinical evidence, it is necessary to carefully examine pediatric patients because the incidence of serious bacterial infection (SBI) in infants younger than 90 days of age has increased by 15 percent (2). Young infants, especially neonates, have decreased antibody activity, macrophage and neutrophil function, and bone marrow insufficiency (3). Therefore, early recognition and treatment of SBI in young infants is important. Various protocols such as Rochester, Philadelphia, and Boston criteria have been developed to prevent unnecessary treatment and hospitalization. Although these protocols include invasive procedure such as lumbar puncture, they may miss SBI. Moreover, these protocols do not include neonates because they are not for use in this population (4). All febrile neonates are advised to receive empirical antibiotics and be hospitalized with sepsis testing (5). Therefore, it is clinically necessary to determine the disposition of febrile infants aged 29 to 90 days.

Previous studies have assessed the usefulness of markers such as white blood cell (WBC) count, absolute neutrophil count (ANC), C-reactive protein (CRP) level, procalcitonin (PCT) level, and neutrophil-to-lymphocyte ratio (NLR) for the prediction of SBI in febrile infants (2, 6). However, to our knowledge, no study has compared these markers together.

The purpose of this study was to compare these markers to identify which can predict SBI in febrile infants aged 29 to 90 days and to determine the cutoff values of these markers.

MATERIALS AND METHODS

Study population

This retrospective cohort study included patients who visited a tertiary hospital emergency department in Korea between July 2016 and June 2018. Infants aged 29 to 90 days with fever above 38°C at home or on admission were enrolled. Patients who were administered antibiotics 48 hours before visiting the hospital or who had a major underlying disease such as immune deficiency, congenital abnormality, or chronic disease were excluded. Blood count, CRP, PCT, and blood and urine cultures were performed on the admitted patients. Patients without PCT results were excluded. SBI was defined as bacterial meningitis, bacteremia, or urinary tract infection (UTI) in which known pathogens were cultured.

Data collection

The following data were collected from the patient medical records: history, physical examination results, laboratory test findings including complete blood count (CBC); CRP level; PCT level; and the results of blood, urine, and cerebrospinal fluid (CSF) cultures. All laboratory tests were performed within 12 hours of visiting the emergency department. The CBC was measured using an automated cell counter and the CRP level was measured using a routine automated analyzer (Hitachi Clini-

cal Analyzer 7600). The PCT levels were quantitated by immunoassay (Roche Elecsys E170).

The ANC was obtained from the CBC and the NLR was calculated. UTI was defined as the isolation of >50,000 colony-forming units per milliliter of urine of a single pathogen. The culture of two or more pathogens was regarded as contamination. The detection of Staphylococcus epidermidis or Streptococcus viridans in blood or CSF cultures was also considered contamination (2). All SBI cases were reviewed by two emergency medicine physicians.

Sample size

The sample size was calculated by the area under the curve (AUC) of receiver operating characteristic (ROC) curves of the NLR. Assuming an AUC of 0.7 and an SBI prevalence of 10 percent, the required sample size was 190.

Statistical analysis

Continuous variables were evaluated for normal distributions using Shapiro-Wilk tests and histograms. Categorical variables were compared by $\chi 2$ or Fisher exact tests, and continuous variables were compared by t- or Mann-Whitney tests, as appropriate. The cutoff values were calculated by Youden's index, maximum (sensitivity + specificity − 1), based on the ROC curves. The sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, positive predictive value (PPV), and negative predictive value (NPV) value were assessed for the optimal cutoffs obtained from our ROC curve analysis and other previously published cutoffs. The statistical analysis was performed using IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, New York).

RESULTS

A total of 528 infants aged 29 to 90 days were admitted to the emergency department during the study period. Of these, 234 were enrolled for final analysis after excluding those who were preterm (n=27), with underlying disease, and with incomplete records (n=173). Incomplete records include the absence of blood culture or PCT results or inaccurate medical charts. Among the 234 who satisfied the inclusion criteria, 35 with contaminated cultures were excluded to reduce the misclassification bias. Finally, 199 patients were enrolled. (Figure 1)

Of the 199 febrile infants, 68 (34.2%) were diagnosed with SBI. There was no difference in body temperature between the SBI and non-SBI groups. All blood test marker values were significantly higher in the SBI group than those in the non-SBI group: WBC (12.72 vs. 12.72 k/μL, P <0.001), NLR (1.29 vs. 0.78, P <0.001), ANC (6.28 vs. 3.14 k/μL, P <0.001), CRP (2.66 vs 0.28 mg/L, p<0.001), and PCT (0.5 vs. 0 ng/m, P <0.001) (Table 1). Of the 68 infants with SBIs, 63 (92.6%) had UTIs, four (5.9%) had bacteremia, and one (1.5%) had bacterial meningitis. The most common pathogens in the UTIs were Escherichia coli (n=60, 95.2%) (Table 2).

Regression was done by univariate and multivariate analysis based on the clinical and laboratory data. The cutoff values determined by Youden's index for NLR, PCT, CRP, ANC, and WBC were 0.67, 0.6 ng/mL, 16.74 mg/L, 4 k/uL, and 14.47k/uL, respectively. NLR, PCT, CRP, and WBC were risk factors for SBI (Table 3).

In ROC curve analysis, CRP was the best marker for detecting SBI, followed by ANC and NLR (Figure 2). The performances of NLR, PCT, CRP, ANC, and WBC at the selected and standard thresholds are summarized in Table 4. There were six cases (8.8%) of SBI with an NLR of 0.67 or below, 21 (30.9%) with a CRP level of 16.74 mg/L or lower, and 41 (60.3%) with a PCT level of 0.5 ng/mL or lower.

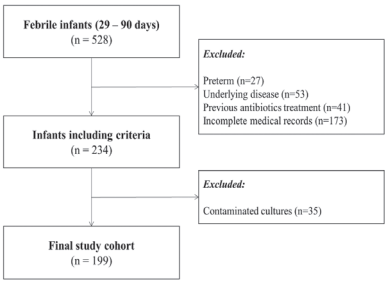
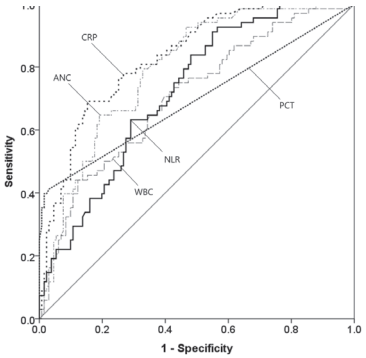


Figure 1. Study population



Biomarker	AUC (95% CI)
NLR	0.722 (0.651 – 0.792)
PCT	0.695 (0.611 – 0.780)
CRP	0.832 (0.775 – 0.889)
ANC	0.793 (0.731 – 0.856)
WBC	0.705 (0.629 – 0.781)

NLR, neutrophil to lymphocyte ratio; PCT, procalcitonin; CRP, C-reactive protein; ANC, absolute neutrophil count; WBC, white blood cell

Figure 2. Receiver operating characteristic curves of biomarkers for the detection of SBI

DISCUSSION

In children, especially young infants, bacterial infection remains a significant cause of mortality and morbidity and, therefore, should be carefully diagnosed and treated (7). Failure to detect bacterial pathogens early may delay the onset of treatment and cause severe illness. Moreover, unnecessary treatment or extended treatment periods may occur and resistant pathogens develop. Although the optimal combination of markers for the detection of SBI in young infants has not yet been determined, its diagnosis should include a variety of clinical and laboratory parameters (8). To the best of our knowledge, this study is the first to compare various markers including NLR and PCT to detect SBI in febrile young infants.

In 2009, Olaciregui et al. (9) reported PCT, CRP, and leukocyte to be predictors of SBI in children less than three months of age and that the diagnostic value of PCT in invasive bacterial infection (IBI), defined as bacteremia or bacterial meningitis, is superior to that of CRP. In 2012, Gomez et al. (10) reported the good diagnostic accuracies of CRP and PCT to detect SBI and that PCT is best for identifying performance in IBI. In addition, Bilavsky et al. found that CPR was a valuable laboratory test for the assessment of febrile infants aged <3 months (11). In our study, young febrile infants with SBI had statistically significant higher mean values of WBC, ANC, NLR, PCT, and CRP. Of these markers, PCT had the highest odds ratio for SBI.

According to Maniaci et al. (12), PCT is the best predictive parameter of SBI in young febrile patients; however, they did not assess CRP. In our study, the odds ratios of PCT were the highest in correlation analysis but its diagnostic accuracy for SBI was not as good as that of CRP in ROC curve analysis (AUC for CRP and PCT 0.832 and 0.695, respectively) Previous studies have reported that the AUC of CRP is equal to or higher than that of PCT in SBI, but that the AUC of PCT is highest in IBI (2, 9, 10, 13). CBC and CRP are generally available in most hospitals, but the measurement of

Table 1. Comparison of laboratory data in patients with and without serious bacterial infection (SBI)

Characteristic	All patients (n = 199)	SBI (n = 68)	non-SBI (n = 131)	P-value
Age (days)	62.0 (44.0-79.0)	65.0 (49.5-83.5)	61.0 (43.0-76.0)	0.172
Body temp (°C)	38.20 (37.80-38.50)	38.25 (37.82-38.80)	38.20 (37.80-38.70)	0.177
WBC (k/μL)	10.6 (7.9-14.19)	12.72 (10.08-17.79)	9.91 (6.94-12.47)	<0.001
NLR	1.03 (0.55-1.51)	1.29 (0.94-1.85)	0.78 (0.48-1.31)	<0.001
ANC (k/μL)	3.96 (2.67-6.61)	6.28 (4.13-9.10)	3.14 (1.93-4.88)	<0.001
CRP (mg/L)	7.3 (2.0-25.8)	26.6 (11.5-52.7)	2.8 (0.9-12.0)	<0.001
PCT (ng/mL)	0 (0-1)	0.5 (0.00-1.28)	0 (0-0)	<0.001

SBI, serious bacterial infection; WBC, white blood cell; NLR, neutrophil to lymphocyte ratio; ANC, absolute neutrophil count; CRP, C-reactive protein; PCT, procalcitonin; Mann-Whitney U test was used

Table 2. Bacterial infections

Infection Type	No. of study participants	Pathogens
SBI	68	
UTI	63	Escherichia coli (n = 60) Klebsiella (n = 2) Enterobacter (n = 1)
Bacteremia	4	Escherichia coli (n = 3) Enterococcus (n = 1)
Bacterial meningitis	1	Escherichia coli (n = 1)
Non-SBI	131	

SBI, serious bacterial infection; UTI, urinary tract infection

Table 3. Clinical and laboratory predictors of patients with serious bacterial infections

Predictor	Crude OR (95% CI)	P-value	AOR (95% CI)	P-value
Temperature≥39°C in ED	1.91 (0.80–4.60)	0.147	0.70 (0.21-2.30)	0.552
NLR ≥ 0.67	8.47 (3.42–20.95)	<0.001	2.89 (1.05-7.94)	0.040
PCT ≥ 0.6 ng/mL	42.48 (9.68–186.35)	<0.001	17.33 (3.54-84.78)	<0.001
CRP ≥ 16.74 mg/L	12.42 (6.16-25.04)	<0.001	5.33 (2.36-12.06)	<0.001
WBC ≥ 14.47 k/μL	5.67 (2.79-11.53)	<0.001	2.61 (1.06-6.42)	0.036

OR, odds ratio; AOR, adjusted odds ratio; ED, emergency department; NLR, neutrophil to lymphocyte ratio; PCT, procalcitonin; CRP, C-reactive protein; WBC, white blood cell; Cutoff points of laboratory tests were calculated as Euclidean method using receiver operating characteristic curve analysis. ANC is excluded to avoid the multicollinearity.

Table 4. Sensitivity, specificity, and likelihood ratio values for serious bacterial infection at various thresholds

Parameter	Threshold	Sensitivity	Specificity	LR+	LR-	PPV	NPV
NLR	>0.67	91.18 (81.8 - 96.7)	45.04 (36.3 - 54.0)	1.66 (1.4 - 2.0)	0.2 (0.09 - 0.4)	46.3	90.8
	>1	70.59 (58.3 - 81.0)	58.78 (49.8 - 67.3)	1.71 (1.3 - 2.2)	0.5 (0.3 - 0.7)	47.1	79.4
	>1.5	38.24 (26.7 - 50.8)	80.15 (72.3 - 86.6)	1.93 (1.2 - 3.0)	0.77 (0.6 - 0.9)	50	71.4
	>2	22.06 (12.9 - 33.8)	90.08 (83.6 - 94.6)	2.22 (1.1 - 4.4	0.87 (0.8 - 1.0	53.6	69
	>3	7.35 (2.4 - 16.3)	98.47 (94.6 - 99.8)	4.82 (1.0 - 24.2)	0.94 (0.9 - 1.0)	71.4	67.2
PCT	>0.5	39.71 (28.0 - 52.3)	97.71 (93.5 - 99.5)	17.34 (5.5 - 55.1)	0.62 (0.5 - 0.7)	90	75.7
	>1	35.29 (24.1 - 47.8)	99.24 (95.8 - 100.0)	46.24 (6.4 - 334.5)	0.65 (0.5 - 0.8)	96	74.7
CRP	>16.74	66.18 (53.7 - 77.2)	84.73 (77.4 - 90.4)	4.33 (2.8 - 6.7)	0.4 (0.3 - 0.6)	69.2	82.8
	>20	61.76 (49.2 - 73.3)	87.79 (80.9 - 92.9)	5.06 (3.1 - 8.3)	0.44 (0.3 - 0.6)	72.4	81.6

	>40	30.88 (20.2 - 43.3)	95.42 (90.3 - 98.3)	6.74 (2.9 - 15.9)	0.72 (0.6 - 0.9)	77.8	72.7
ANC	>4	79.41 (67.9 - 88.3)	67.18 (58.4 - 75.1)	2.42 (1.8 - 3.2)	0.31 (0.2 - 0.5)	55.7	86.3
	>7	44.12 (32.1 - 56.7)	88.55 (81.8 - 93.4)	3.85 (2.2 - 6.7)	0.63 (0.5 - 0.8)	66.7	75.3
	>10	26.47 (16.5 - 38.6)	95.42 (90.3 - 98.3)	5.78 (2.4 - 13.9)	0.77 (0.7 - 0.9)	75	71.4
	>15	1.47 (0.04 - 7.9)	99.24 (95.8 - 100.0)	1.93 (0.1 - 30.3)	0.99 (1.0 - 1.0)	50	66
WBC	>10	75 (63.0 - 84.7)	52.67 (43.8 - 61.5)	1.58 (1.3 - 2.0)	0.47 (0.3 - 0.7)	45.1	80.2
	>15	39.71 (28.0 - 52.3)	87.79 (80.9 - 92.9)	3.25 (1.9 - 5.6)	0.69 (0.6 - 0.8)	62.8	73.7
	>20	16.18 (8.4 - 27.1)	96.95 (92.4 - 99.2)	5.3 (1.8 - 16.0)	0.86 (0.8 - 1.0)	73.3	69

LR, Likelihood ratio; PPV, positive predictive value; NPV, negative predictive value; NLR, neutrophil to lymphocyte ratio; PCT, procalcitonin; CRP, C-reactive protein; ANC, absolute neutrophil count; WBC, white blood cell

PCT is not possible in some hospitals and is more expensive than CRP. Therefore, CRP is considered sufficient to detect SBI.

In the United States, the incidence of IBI in full-term infants younger than 90 days is 37.5 percent (14). Recent studies reported a 10-11 percent incidence in febrile young infants (6, 13). In our study, the incidence of SBI was 34.2 percent; the reason for the higher incidence may have been that the patients' conditions may have been more serious because we enrolled only those who visited the emergency department.

UTI is the most common bacterial infection requiring antibiotic treatment in pediatric fever (15). UTI is also the most frequent cause of SBI, accounting for 80 percent of all SBIs (2). In our study, the incidence of UTI was 31.7 percent, accounting for 92.6 percent of the SBI cases. The incidence of UTI may be variable depending on the sample collection method. In one study in which only catheterization was used for urine collection, UTI accounted for 5.6 percent (16). Another study observed that 14.1 percent of samples from catheterization and bag were positive for UTI (10). Urine collection from catheterization is perhaps the best method in infants. However, the urine bag is still used to collect urine. Urine bags were also used in most patients in our study. A previous study reported a 1 percent incidence of bacteremia and 0.6 percent of bacterial meningitis (2). The incidence in the present study was 2 percent (n=4) and 0.5 percent (n=1), respectively.

The diagnostic properties through threshold values based on cutoffs using Youden's index and traditional cutoffs result in high sensitivity (91.18%) and negative predictive value (90.8%) for an NLR < 0.67. Planning discharge in emergency department is important. Until now, emergency physicians used protocols for safe discharge such as the Rochester, Philadelphia, and Boston criteria (17-19). Combining the NLR with these protocols will result in safer patient discharge in young febrile infants.

This study has several limitations. First, the evaluation of cohorts was retrospective and there was unavoidable selection bias. However, the hospital where this study was carried out has a "critical pathway for febrile children under 90 days"; therefore, the emergency physicians performed diagnostic tests and treated the patients in the same way, including blood and urine cultures and PCT assessment. Therefore, the selection bias was minimized. Second, bacterial infections such as bacterial pneumonia, gastroenteritis and arthritis, were not included. However, these infections are fairly rare in this age group. Third, we did not distinguish IBI from SBI because only five patients had bacteremia or meningitis.

No laboratory parameters can replace clinical judgment. Clinical findings such as history taking and physical examination are the most important and laboratory tests are an ancillary tool. Febrile children with a toxic appearance should be hospitalized. The results of this study will help in decision-making in young febrile infants with non-toxic appearance. An NLR below 0.67 can help decide discharge. A CRP level of 16.74 mg/L can help decide admission.

CONCLUSIONS

In young febrile infants, CRP was the best single discriminatory marker of SBI. Additionally, the NLR was the best parameter for considering discharge. However, it is necessary to consider the combination of clinical features and laboratory finding for decision-making on young febrile infants in the emergency department.

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