




# Predictors of serious bacterial infections using serum biomarkers in an infant population aged 0 to 90 days: a prospective cohort study

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## ABSTRACT

**Objective** Young febrile infants represent a vulnerable population at risk for serious bacterial infections (SBI). We aimed to evaluate the diagnostic accuracy of components of the complete blood count in comparison with C-reactive protein (CRP) to predict SBI among febrile infants.

**Design and setting** Prospective cohort study conducted in a tertiary emergency department between December 2018 and November 2019.

**Patients** We included febrile infants ≤3 months old with complete blood count results. We analysed their white blood cell count (WBC), absolute neutrophil ratio (ANC), neutrophil to lymphocyte ratio (NLR), platelet to lymphocyte ratio, mean platelet volume to platelet count ratio, and compared these to the performance of CRP.

**Main outcome measures** SBIs were defined as urinary tract infection, bacteraemia, bacterial meningitis, sepsis, pneumonia, skin and soft tissue infection, bacterial enteritis, septic arthritis or osteomyelitis.

**Results** Of the 187 infants analysed, 54 (28.9%) were diagnosed with SBI. Median values of WBC, ANC, NLR and CRP were significantly higher in infants with SBI: WBC (13.8 vs 11.4×10<sup>9</sup>/L, p=0.004), ANC (6.7 vs 4.1×10<sup>9</sup>/L, p<0.001), NLR (1.3 vs 0.9, p=0.001) and CRP (21.0 vs 2.3 mg/L, p<0.001), compared with those without. CRP had the best discriminatory values for SBI, with area under the curve (AUC) of 0.815 (95% CI 0.747 to 0.883), compared with WBC, ANC and NLR. A predictive model consisting of WBC, ANC and NLR in combination with clinical parameters, had an AUC of 0.814 (95% CI 0.746 to 0.883). There was increased discriminative performance when this predictive model was combined with CRP, (AUC of 0.844, 95% CI 0.782 to 0.906).

**Conclusion** In young febrile infants, CRP was the best discriminatory biomarker for SBI. WBC, ANC and NLR when used in combination have potential diagnostic utility in this population.

## INTRODUCTION

Febrile infants under 3 months of age represent a significant proportion of annual emergency department (ED) attendances.<sup>1</sup> An estimated 10% of febrile infants under 60 days will have serious bacterial infections

## What is known about the subject?

- Identifying young febrile infants at risk for serious bacterial infections is a significant diagnostic challenge.
- Biomarkers including white blood cell count, absolute neutrophil count, C-reactive protein and procalcitonin are frequently evaluated in clinical prediction models.
- Other aspects of the complete blood count including neutrophil to lymphocyte ratio, platelet to lymphocyte ratio and mean platelet volume to platelet count ratio have been described as promising predictive biomarkers of sepsis in adults, but have not been widely studied in the infant population.

## What this study adds?

- C-reactive protein (CRP) remains the most valuable discriminating biomarker for serious bacterial infections among young febrile infants.
- In the absence of CRP, low cost and readily available biomarkers, including white blood cell count, absolute neutrophil ratio and neutrophil to lymphocyte ratio, when used in combination have potential diagnostic value in the evaluation of this population of febrile infants.

(SBI), including urinary tract infection (UTI), bacteraemia and bacterial meningitis.<sup>1–3</sup> Invasive bacterial infections (IBI) are commonly defined as the presence of bacteraemia and bacterial meningitis.<sup>4–6</sup> SBIs and IBIs, if not promptly treated, can result in mortality, long-term cognitive deficits and hearing loss.<sup>7,8</sup> While prompt identification and treatment of sepsis in this group is crucial, these young infants often present with vague symptoms without a clear focus of infection. Clinicians are faced with the diagnostic dilemma of whether the infant has an

SBI or a self-resolving viral infection. They often end up routinely performing invasive investigations such as blood sampling and lumbar puncture, and have a low threshold to start empirical broad spectrum antibiotics.

Biomarkers that have been studied include white blood cell count (WBC), absolute neutrophil count (ANC), C-reactive protein (CRP) and procalcitonin (PCT). These markers have been evaluated in clinical prediction rules, used often in combination or together with clinical findings, to risk stratify febrile infants.<sup>9–12</sup> However, PCT is not widely available in many settings owing to cost, and has been reported to have wide variations in its sensitivity and specificity.<sup>13 14</sup>

There has been increasing interest in different aspects of the complete blood count as an easily available and low-cost test. Neutrophil to lymphocyte ratio (NLR) was first reported by Zahorec to reflect the intensity of the immune response to systemic inflammation, surgical stress or sepsis in critically ill adult patients.<sup>15</sup> In adult studies, it can predict the presence of bacteraemia and poor outcomes in patients with sepsis.<sup>16–18</sup> A few studies have found NLR to be valuable in diagnosing paediatric and neonatal sepsis, alongside other widely used blood markers including ANC and CRP.<sup>4 19 20</sup>

Platelet and lymphocytes derangements are also measures of systemic inflammation. Mean platelet volume to platelet count ratio (MPV/PC) has been suggested in adult studies as an early prognostic marker of outcome in critically ill states secondary to sepsis or trauma.<sup>18 21</sup> Platelet to lymphocyte ratio (PLR) is another novel biomarker, reflecting the balance between inflammation and thrombosis. It has been shown to be useful in adult studies to diagnose inflammatory conditions.<sup>18 22</sup> However, these markers have not been as widely studied in the paediatric population.

The primary aim of our study was to investigate the diagnostic accuracy of these easily available biomarkers to predict SBI: WBC, ANC, NLR, PLR, MPV/PC and to compare them with CRP in febrile infants less than 3 months old. Our secondary aim was to determine cut-off values for these biomarkers that would be useful to risk stratify young febrile infants.

## METHODS

### Study design and setting

We performed a prospective observational cohort study, between December 2018 and November 2019, at the ED of KK Women's and Children's Hospital (KKH) in Singapore. KKH is the larger of two tertiary paediatric centres in Singapore, with an annual ED attendance of approximately 150 000 children, aged between 0 to 17 years of age.

### Study population

We recruited infants if they met the following inclusion criteria: (1)  $\leq 90$  days of age, and (2) presented with fever, defined as an axillary or rectal temperature of  $\geq 38^{\circ}\text{C}$

either at triage (measured by the triage nurse) or during consultation (measured by the doctor), when wrapped in a single layer. We excluded (1) infants without complete blood count results, (2) preterm infants  $< 35$  weeks gestation, (3) infants with perinatal asphyxia or significant neonatal complications requiring prolonged stay ( $> 7$  days) in the neonatal intensive care unit, (4) those who received antibiotics in the preceding 48 hours and (5) those with underlying haematological or immunological disease. In our hospital, all febrile infants younger than 3 months are hospitalised, forming a natural pool with complete outcomes documented. Subsequently, the type and extent of investigations are decided by the ward physicians. It is usual practice for neonates less than 28 days old with fever to undergo blood, urine and cerebrospinal fluid (CSF) cultures. However, older infants may undergo more limited investigations depending on clinical assessment. All infants are monitored in hospital and are reviewed by medical doctors twice a day or more depending on their clinical status, until the fever has resolved for at least 24 hours before they are discharged.

### Definitions and outcome measures

We classified infants with serious bacterial infection as (1) UTI, defined by the growth of a single organism with (a) at least 50 000 colony-forming units (CFU)/mL from a catheterised urine specimen, (b) 10 000 to 50 000 CFU/mL from a catheterised specimen in association with an abnormal urinalysis (positive for leucocyte esterase, nitrite or pyuria with  $> 5$  white blood cells per high-power-field on urine microscopy) or (c) at least 100 000 CFU/mL from urine collected via a voided specimen,<sup>23 24</sup> (2) bacteraemia, defined by the growth of a single bacterial pathogen in the blood, excluding growth of bacteria considered to be likely contaminants such as coagulase-negative staphylococcus,<sup>9 25</sup> (3) bacterial meningitis, defined by the growth of bacteria in the CSF,<sup>26 27</sup> (4) sepsis defined as documented or suspected infection and findings of inflammation such as haemodynamic instability, tissue perfusion alteration and/or indications of end-organ dysfunction,<sup>28</sup> that demonstrated clinical improvement after fluid resuscitation and early antibiotic administration, (5) pneumonia defined on chest X-ray as reported by a trained radiologist, (6) bacterial enteritis confirmed by a positive stool culture, (7) infection of skin and soft tissues (cellulitis or similar), diagnosed by suggestive physical examination findings or (8) osteomyelitis or septic arthritis. In addition, we included in the definition of bacterial meningitis, infants with either (1) bacteraemia with an organism known to cause central nervous system infection and sterile CSF pleocytosis,<sup>26 27</sup> or (2) sterile CSF pleocytosis with biochemical changes consistent with bacterial meningitis including CSF to plasma glucose ratio of less than 0.6, with no other attributable source of infection. For cases where chart review was unclear, a consensus was reached by consulting the infectious diseases specialist. CSF pleocytosis was defined as (1) CSF white blood cells  $\geq 20$  cells/uL for infants

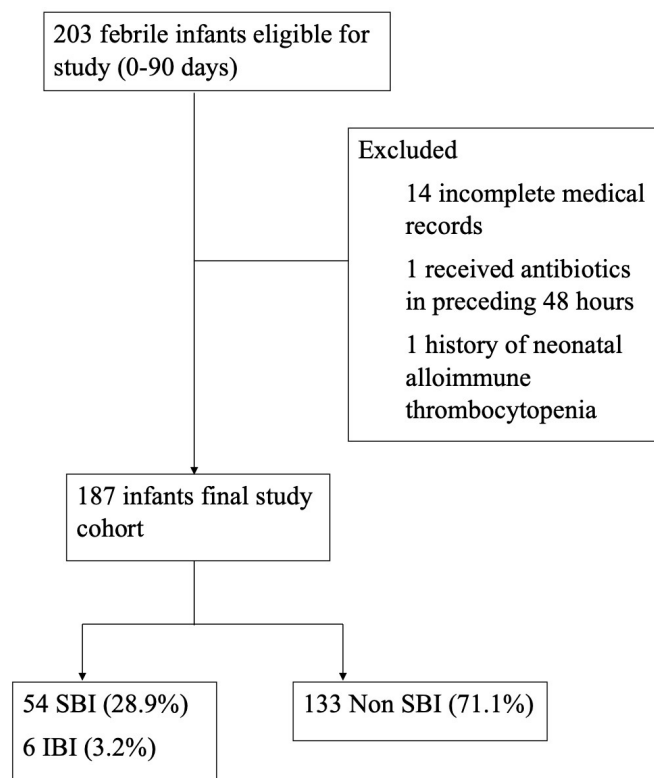
≤28 days or ≥10 cells/uL for infants 29 to 90 days of age with (2) CSF red blood cells <100 cells/uL.<sup>26 27</sup> IBI was defined by bacteraemia or bacterial meningitis.<sup>4-6</sup> Infants were included in the non-SBI group if they had: (1) negative cultures or (2) demonstrated clinical improvement defined as resolution of fever and symptoms without a complete workup. The final diagnoses were determined after an independent review of the patients' clinical course and investigations by a trained clinician.

We obtained demographic data, clinical history and physical examination, laboratory investigations including complete blood count, CRP, PCT, blood cultures, urine cultures and lumbar puncture investigation results from the electronic medical records. The complete blood count was determined using an automated haematology analyser (Sysmex XN-1000 and XN-3000, Kobe, Japan). Total white cell count with differential count was measured using flow cytometry method, while platelet count was determined using impedance method. CRP measurement was performed using a quantitative immunoturbidimetric assay (Alinity c analyser, Abbott Laboratories, Italy). From the complete blood count, we retrieved data including WBC and platelet count, ANC, absolute lymphocyte count and MPV. NLR was calculated as the ratio of neutrophils to lymphocytes, PLR as the ratio of platelets to lymphocytes and MPV/PC as the ratio of MPV to platelet count.

### Statistical analysis

All data were entered into a Microsoft Excel database. Statistical analysis was performed using IBM SPSS Statistics, V.26 and SAS software, V.9.4 (SAS Institute Inc). At baseline, categorical variables were summarised as counts and percentages (%), and continuous variables as mean and SD or median and IQR. Continuous variables were assessed for normality using q-q plots and histograms. After applying a normalising log transformation on variables with right-skewed distributions, the normality assumption was found to be tenable. Statistical comparisons between groups on normal and normalised variables were performed using a two sample t-test. In addition, variables with right-skewed distributions were compared using a Wilcoxon rank-sum test. Categorical variables were compared using Fisher's exact test.

We performed a univariate analysis for each potential predictor and presented these using unadjusted OR estimates, with 95% CIs. In the multivariable analyses, we studied the performance of the specific variables of interest that were statistically significant on univariate analysis (complete blood count indices WBC, ANC and NLR) and adjusted for age of the infant, gender and duration of fever before presentation. We derived predictive models with and without CRP to address the primary aim of the paper. Adjusted OR (aOR) were presented with their corresponding 95% CIs and statistical significance was taken at  $p < 0.05$ . We presented discriminative ability using the area under the receiver operating characteristic (ROC) curve (AUC) analyses and optimal cut-off



**Figure 1** Patient flow diagram. IBI, invasive bacterial infections; SBI, serious bacterial infections.

values were calculated using the Youden's index (sensitivity+specificity -1). We compared the area under the ROC curves for significant differences, using DeLong's method.<sup>29</sup>

### RESULTS

Among 203 febrile infants recruited, 187 were included in the final analysis (figure 1). There were no significant differences in baseline characteristics between the included and excluded patients in this study (online supplemental eTable 1).

Only one patient was discharged against medical advice and all other infants were hospitalised. Seventeen (9.1%) infants received intravenous fluid bolus in the ED, while 1 (0.5%) required intensive care. The majority had urine cultures (158, 84.4%), blood cultures (153, 81.8%) and lumbar punctures for CSF analysis (121, 64.7%). Most received intravenous antibiotics (153, 81.8%).

Table 1 describes the demographic and clinical characteristics of the SBI and non-SBI group. The overall median age at presentation was 35 days (IQR 9 to 61). Thirty-six (19.3%) infants were born to mothers with Group B *Streptococcus* (GBS) colonisation, among whom 35 (97.2%) received treatment for GBS.

Mean and median values of WBC, ANC, NLR and CRP were significantly higher in infants with SBI compared with those without (table 1). When comparing infants with IBI to those without IBI, only PLR was significantly higher: PLR (149.7, IQR 88.2 to 250.5, vs 86.0, IQR 68.6

**Table 1** Clinical and laboratory characteristics of infants with and without serious bacterial infections

Variable	SBI (n=54)	Non SBI (n=133)	Overall (n=187)	P value
Age at presentation				0.016
0 to 28 days, n (%)	14 (25.9)	65 (48.9)	79 (42.2)	
29 to 60 days, n (%)	22 (40.7)	38 (28.6)	60 (32.1)	
61 to 90 days, n (%)	18 (33.3)	30 (22.6)	48 (25.7)	
Age in days, median (IQR)	48 (28 to 69)	29 (6 to 56)	35 (9 to 61)	0.004
mean (SD)	45 (24.5)	33 (28.3)	37 (27.7)	<0.001*
Gender				
Male, n (%)	43 (79.6)	66 (49.6)	109 (58.3)	<0.001
Ethnicity, n (%)				0.180
Chinese	35 (64.8)	66 (49.6)	101 (54.0)	
Malay	12 (22.2)	50 (37.6)	62 (33.2)	
Indian	3 (5.6)	10 (7.5)	13 (7.0)	
Others	4 (7.4)	7 (5.3)	11 (5.9)	
Maternal positive GBS status, n (%)	12 (22.2)	24 (18.0)	36 (19.3)	0.511
<24 hours of fever at presentation, n (%)	38 (70.4)	117 (88.0)	155 (82.9)	0.004
Comorbidities, n (%)	3 (5.6)	2 (1.5)	5 (2.7)	0.120
Length of hospital stay in days, median (IQR)	4 (3 to 4)	3 (3 to 4)	3 (3 to 4)	0.015
Temperature °C, mean (SD)	38.7 (0.6)	38.5 (0.5)	38.6 (0.6)	0.051
Heart rate per minute, mean (SD)	168 (26)	164 (19)	166 (21)	0.318
Respiratory rate per minute, mean (SD)	42 (6)	41 (6)	41 (6)	0.216
Pulse oximetry %, median (IQR)	99 (98 to 100)	99 (98 to 100)	99 (98 to 100)	0.495
WBC, 10 <sup>9</sup> /L, median (IQR)	13.8 (8.5 to 19.1)	11.4 (8.5 to 13.6)	11.8 (8.5 to 14.4)	0.004
mean (SD)	14.1 (6.4)	11.3 (4.4)	12.1 (5.2)	0.017*
ANC, 10 <sup>9</sup> /L, median (IQR)	6.7 (3.1 to 9.5)	4.1 (2.6 to 5.9)	4.5 (2.9 to 6.9)	<0.001
mean (SD)	7.2 (4.4)	4.6 (3.0)	5.4 (3.6)	<0.001*
NLR, median (IQR)	1.3 (0.9 to 1.9)	0.9 (0.6 to 1.5)	1.0 (0.6 to 1.6)	0.001
mean (SD)	1.6 (1.0)	1.1 (0.8)	1.2 (0.9)	0.001*
PLR, median (IQR)	86.7 (68.8 to 138.6)	86.9 (69.5 to 14.4)	86.9 (68.8 to 120.1)	0.878
mean (SD)	105.9 (53.4)	107.1 (73.1)	106.7 (67.8)	0.703*
MPV/PC, median (IQR)	0.11 (0.07 to 0.15)	0.12 (0.09 to 0.15)	0.12 (0.08 to 0.15)	0.768
mean (SD)	0.12 (0.05)	0.13 (0.07)	0.12 (0.06)	0.634*
	<b>SBI (n=54)</b>	<b>Non SBI (n=125)</b>	<b>Overall (n=179)</b>	
CRP, mg/L, median (IQR)	21.0 (7.2 to 48.7)	2.3 (1.0 to 6.8)	3.8 (1.0 to 17.1)	<0.001
mean (SD)	30.2 (30.1)	7.5 (16.7)	14.3 (23.9)	<0.001*

\*P values performed on log-transformed data.

ANC, absolute neutrophil ratio; CRP, C-reactive protein; GBS, Group B *Streptococcus*; MPV/PC, mean platelet volume to platelet count ratio; NLR, neutrophil to lymphocyte ratio; PLR, platelet to lymphocyte ratio; SBI, serious bacterial infections; WBC, white blood cell count.

to 114.4,  $p=0.038$ ). The median value of MPV/PC did not differ significantly when comparing infants with SBI and IBI to those without ( $p=0.768$  and  $p=0.065$  for SBI and IBI, respectively).

Fifty-four infants (28.9%) were diagnosed with SBI, and 6 (3.2%) with IBI. UTI was the most common SBI (36, 66.7%), of which *Escherichia coli* (29, 80.6%) was the most common pathogen. The second most common SBI was sepsis (12, 22.2%). The main causative organism for bacteraemia was GBS, while cases of meningitis were largely secondary to *Klebsiella pneumoniae*, *Escherichia coli* and GBS. The prevalence of each SBI in various age groups is detailed in [table 2](#).

WBC, ANC, NLR and CRP were significantly associated with SBI in the univariate analysis ([table 3](#)). In the multivariable analysis without CRP, WBC (aOR 0.730, 95% CI 0.540 to 0.986) and ANC (aOR 2.318, 95% CI 1.245 to 4.315) were significant. However, when CRP was added to the model, only ANC (aOR 1.934 (95% CI 1.007 to 3.714) and CRP (aOR 1.037, 95% CI 1.013 to 1.063) were statistically significant.

CRP had the best discriminatory values for SBI, with AUC 0.815 (95% CI 0.747 to 0.883), followed by ANC and NLR, with AUC 0.681 (95% CI 0.587 to 0.775) and AUC 0.656 (95% CI 0.571 to 0.740), respectively ([table 4](#)). Sensitivities, specificities, likelihood ratios and positive

**Table 2** Diagnoses of serious bacterial infections in each age group

	Serious bacterial infection (n=54/187)	Infants aged 0 to 28 days (n=14/79)	Infants aged 29 to 60 days (n=22/60)	Infants aged 61 to 90 days (n=18/48)
Urinary tract infection, n (%)	36 (66.7)	9 (64.2)	14 (63.6)	13 (72.2)
Sepsis, n (%)	12 (22.2)	1 (7.1)	5 (22.7)	6 (33.3)
Bacterial meningitis, n (%)	4 (7.4)	1 (7.1)	1 (4.5)	2 (11.1)
Bacteraemia, n (%)	4 (7.4)	1 (7.1)	1 (4.5)	2 (11.1)
Pneumonia, n (%)	3 (5.6)	2 (14.3)	1 (4.5)	0
Skin and soft tissue infection, n (%)	2 (3.7)	1 (7.1)	1 (4.5)	0
Bacterial enteritis, n (%)	1 (1.9)	0	1 (4.5)	0
Osteomyelitis, n (%)	0	0	0	0
Two or more listed SBI	6 (11.1)	1 (7.1)	1 (4.5)	4 (22.2)

SBI, serious bacterial infections.

and negative predictive values of the various biomarkers based on optimal cut-offs are also presented in [table 4](#). At the cut-off of 7.2 mg/L, CRP had the highest sensitivity of 75.47% (95% CI 61.72 to 86.24) and negative predictive value of 88.29% (95% CI 68.27 to 92.44) for SBI. Using this optimal cut-off value of CRP, 13 (24.1%) infants with SBI and 2 (33.3%) of infants with IBI were not diagnosed accurately. These missed cases of SBI included 7 (53.8%) infants with urinary tract infection, 1 patient with bacterial meningitis and 1 patient with concomitant GBS bacteraemia and GBS meningitis.

The optimal cut-off values based on Youden's index for WBC, ANC and NLR are  $13.83 \times 10^9/L$ ,  $6.37 \times 10^9/L$  and 1.24, respectively ([table 4](#)). Using clinical parameters with these complete blood count biomarkers (WBC, ANC and NLR) in a predictive model, we derived an AUC of 0.814 (95% CI 0.746 to 0.883). When CRP was added to this predictive model, there was significantly improved discriminative performance with an AUC of 0.844 (95% CI 0.782 to 0.906) ( $p=0.046$ ) ([figure 2](#)).

## DISCUSSION

We described the predictive value of WBC, ANC, NLR, PLR and MPV/PC, and compared these to CRP in discriminating SBI in the young infant population. Our

study shows that CRP had the best performance as a discriminatory biomarker for infants with SBI. Within the complete blood count, ANC performed better than NLR. We found no significant difference in platelet markers including PLR and MPV/PC between the two groups. We demonstrated that although WBC, ANC and NLR did not perform satisfactorily as independent predictors, when used in combination they could potentially value-add to the clinician's armamentarium when predicting SBI. When these biomarkers were used together with CRP, there was improved discriminative performance.

The reported prevalence of SBI in literature varies widely, from 7.1% to 26%.<sup>3 4 9 11 12 30</sup> Our study reports a relatively high prevalence of SBI of 28.9%. This is likely due to our broad case definition of SBI, including clinically significant cases of bacterial infection such as pneumonia, skin and soft tissue infection, bacterial enteritis and sepsis with associated haemodynamic instability that responded to fluid resuscitation and early antibiotic administration. Culture-negative sepsis, in particular in early onset sepsis, is well described in the neonatal population, possibly due to small volumes of blood obtained for culture, low levels of bacteraemia or fastidious bacteria.<sup>31-34</sup> Our study found UTI to be the most common SBI, similar to reported literature.<sup>4 9 11 12</sup>

**Table 3** Univariate and multivariate analysis (with and without CRP) of clinical and laboratory predictors of infants with serious bacterial infections

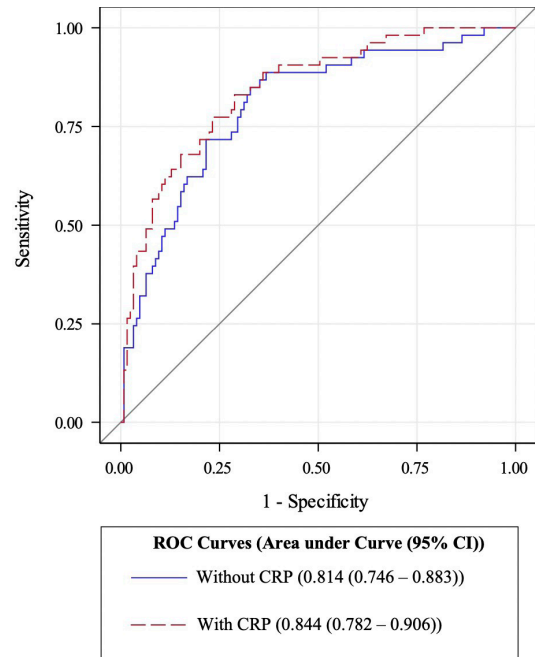
	Univariate analysis		Multivariate analysis without CRP		Multivariate analysis with CRP	
	Unadjusted OR (95% CI)	P value	Adjusted OR (95% CI)	P value	Adjusted OR (95% CI)	P value
Age at presentation	1.016 (1.004 to 1.028)	<b>0.008</b>	1.012 (0.998 to 1.026)	0.099	1.006 (0.991 to 1.021)	0.424
Gender	3.968 (1.885 to 8.354)	<b>0.005</b>	4.049 (1.740 to 9.424)	<b>0.001</b>	3.935 (1.597 to 9.693)	<b>0.003</b>
<24 hour of fever at presentation	0.325 (0.148 to 0.711)	<b>0.005</b>	0.418 (0.165 to 1.062)	0.067	0.978 (0.318 to 3.008)	0.969
WBC	1.108 (1.038 to 1.177)	<b>0.001</b>	0.730 (0.540 to 0.986)	<b>0.040</b>	0.762 (0.559 to 1.039)	0.086
ANC	1.224 (1.100 to 1.347)	<b>&lt;0.001</b>	2.318 (1.245 to 4.315)	<b>0.008</b>	1.934 (1.007 to 3.714)	<b>0.048</b>
NLR	1.631 (1.141 to 2.330)	<b>0.007</b>	0.279 (0.077 to 1.018)	0.053	0.330 (0.085 to 1.282)	0.109
CRP	1.053 (1.030 to 1.074)	<b>&lt;0.001</b>			1.037 (1.013 to 1.063)	<b>0.003</b>

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

**Table 4** Sensitivity, specificity and likelihood ratio values of biomarkers for discrimination of serious bacterial infection at optimal cut-offs

Biomarker	AUC (95% CI)	Optimal cut-off	Sensitivity	Specificity	LR +	LR -	PPV	NPV
WBC, 10 <sup>9</sup> /L	0.632 (0.535 to 0.730)	13.83	50.00%(36.08 to 63.92)	78.95%(71.03 to 85.53)	2.38 (1.55 to 3.63)	0.63 (0.48 to 0.84)	49.09%(38.70 to 59.56)	79.55%(74.60 to 83.74)
NLR	0.656 (0.571 to 0.740)	1.24	57.41%(43.21 to 70.77)	69.17%(60.58 to 76.89)	1.86 (1.32 to 2.62)	0.62 (0.44 to 0.86)	43.08%(34.95 to 51.61)	79.98%(74.18 to 84.75)
ANC, 10 <sup>9</sup> /L	0.681 (0.587 to 0.775)	6.37	53.70%(39.61 to 67.38)	89.27%(82.57 to 92.93)	5.01 (3.21 to 7.81)	0.52 (0.39 to 0.69)	67.05%(56.58 to 76.06)	82.59%(78.01 to 86.38)
CRP, mg/L	0.815 (0.747 to 0.883)	7.2	75.47%(61.72 to 86.24)	75.20%(66.68 to 82.49)	3.04 (2.16 to 4.28)	0.33 (0.20 to 0.53)	55.30%(46.78 to 63.51)	88.29%(82.27 to 92.44)

ANC, absolute neutrophil ratio; AUC, area under the curve; CRP, C-reactive protein; LR, likelihood ratio; NLR, neutrophil to lymphocyte ratio; NPV, negative predictive value; PPV, positive predictive value; WBC, white blood cell count.



**Figure 2** Receiver operating characteristic (ROC) curves for demographic predictors and complete blood count biomarkers with and without C-reactive protein (CRP) for discrimination of serious bacterial infections.

We did find that those with SBI tended to be older, with the highest prevalence reported in the 29 to 60 days age group, which differs from larger studies that report that infants with SBI were more likely to be 28 days or younger.<sup>29</sup>

Screening tools described in literature often use a combination of various blood biomarkers for risk stratification of febrile infants. Recent validated sequential algorithms and clinical prediction rules to aid in identification of young febrile infants at risk of serious bacterial infections often include PCT, which is not routinely available as a point of care test in many settings and is costly.<sup>9 12</sup> Although PCT is known to have superior accuracy as a diagnostic marker for SBI, its reported sensitivity and specificity in literature is variable likely due to heterogeneous study populations and differing cut-off values.<sup>13 14</sup> The use of PCT alone is reported to be inferior to available clinical prediction rules for identifying febrile infants at risk for SBI.<sup>13</sup> We evaluated components of the complete blood count as fast, easily available and relatively low cost biomarkers. Out of these, only WBC, ANC, NLR had statistically significant higher median values for infants with SBI compared with those without. ANC had the best performance with AUC of 0.681 (95% CI 0.587 to 0.775). However, comparison with CRP showed that CRP had significantly better performance as a discriminatory biomarker, with AUC of 0.815 (95% CI 0.747 to 0.883). This finding concurs with previous studies which describes CRP as the most discriminatory biomarker for young infants with SBI, as compared with components of the complete blood count.<sup>4 5 35</sup> The performance of

WBC, ANC and NLR in our study, however, was poorer compared with previously reported studies.<sup>4 35</sup>

In our study, at the optimal cut-off level of 7.2 mg/L, CRP had a reasonably high sensitivity of 75.47% (95% CI 71.72 to 86.24) and negative predictive value 88.29% (95% CI 68.27 to 92.44). This cut-off value is much lower compared with values used in other predictive models for discrimination of SBI in young infants, which are in the range of 20 to 46.1 mg/L.<sup>4 10–12</sup> This may be due to the majority of infants in our cohort presenting early with less than 24 hours of fever, as Singapore is highly urbanised with rapid hospital access. Our study suggests that a lower CRP cut-off might be necessary to distinguish infants with SBI during early onset of symptoms. CRP is known to rise 4 to 6 hours after the onset of symptoms and peak 24 to 48 hours later.<sup>36–38</sup> This is similarly demonstrated in our study, where even at the optimal cut-off level, 13 (24.1%) infants with SBI and 2 (33.3%) of infants with IBI were incorrectly classified. Of note, a 77-day-old infant among these had GBS bacteraemia and GBS meningitis, who presented with an initial CRP of 1.8 mg/L. This further highlights the pitfall of relying on a single biomarker for identifying febrile infants at risk of SBI, especially early in the course of illness.

The value of CRP as a good discriminatory biomarker is demonstrated in our study by the improved performance of a predictive model with clinical parameters and complete blood count biomarkers after its inclusion, with an AUC of 0.844 (95% CI 0.782 to 0.906). Hamiel *et al* similarly found that in the assessment of SBI among young febrile infants, CRP combined with either ANC or NLR offered improved discriminative ability.<sup>4</sup> However, in the absence of CRP, we found that the predictive model with clinical parameters, WBC, ANC and NLR performed with a reasonable AUC of 0.814 (95% CI 0.746 to 0.883). This suggests that there may be clinical utility in combining these indices in the discrimination of SBI, when CRP is not available.

### Strengths and limitations

We performed a prospective study analysing a combination of these biomarkers including NLR, PLR and MPV/PC in this age group, thus adding value to previous retrospective reports.<sup>4 5 35</sup> Unlike most studies looking at only SBIs such as UTI, bacterial meningitis and bacteraemia,<sup>4 9 25 35</sup> we also included other important SBIs in our analysis, such as cases of sepsis, pneumonia, bacterial enteritis and skin infection which have clinical implications.

We recognise the limitations of our study. Not all infants underwent a full evaluation to look for bacteraemia, UTI and meningitis, which may have resulted in potential missed occult bacterial infections. However, we are confident that these numbers are small, since it is our hospital protocol that all young febrile infants undergo strict inpatient monitoring of their temperature and vital parameters for at least 24 hours before discharge. Infants are only allowed to be discharged if they remained well

with no fever or clinical evidence of serious bacterial infection during this period of observation, and after repeated medical assessment. We did not document the timing of vaccinations (if at all) administered to these infants, which may have affected levels of CRP.<sup>39 40</sup> While we tried to adjust for possible confounders through suitable exclusion criteria and with a multivariate analysis, we were not able to adjust for all possible confounders that would affect values of WBC, ANC, NLR and CRP, including maternal fever and causes of stressful delivery. We had small numbers of IBI, resulting in this subpopulation being underpowered for the analysis. Future larger prospective studies will be needed to validate these results, especially among infants with IBI.

### CONCLUSION

In a population of febrile infants under 3 months of age, we found that CRP was the single best biomarker for distinguishing SBI. In the absence of CRP, we demonstrated that low cost and readily available biomarkers including WBC, ANC and NLR can add diagnostic utility when used in combination. There may be a role in the combination of these biomarkers with clinical findings to aid in risk stratification and subsequent management of young febrile infants.

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#### REFERENCES

- Aronson PL, Thurm C, Williams DJ, *et al.* Association of clinical practice guidelines with emergency department management of febrile infants  $\leq 56$  days of age. *J Hosp Med* 2015;10:358–65.
- Powell EC, Mahajan PV, Roosevelt G, *et al.* Epidemiology of bacteremia in febrile infants aged 60 days and younger. *Ann Emerg Med* 2018;71:211–6.
- Greenhow TL, Hung Y-Y, Pantell RH. Management and outcomes of previously healthy, full-term, febrile infants ages 7 to 90 days. *Pediatrics* 2016;138. doi:10.1542/peds.2016-0270. [Epub ahead of print: 01 Nov 2016].
- Hamiel U, Bahat H, Kozler E, *et al.* Diagnostic markers of acute infections in infants aged 1 week to 3 months: a retrospective cohort study. *BMJ Open* 2018;8:e018092.
- Olaciregui I, Hernández U, Muñoz JA, *et al.* Markers that predict serious bacterial infection in infants under 3 months of age presenting with fever of unknown origin. *Arch Dis Child* 2009;94:501–5.
- Herrero M, Alcalde M, Gómez B, *et al.* Invasive bacterial infections in a paediatric emergency department in the era of the heptavalent pneumococcal conjugate vaccine. *Eur J Emerg Med* 2012;19:89–94.
- Byington CL, Reynolds CC, Korgenski K, *et al.* Costs and infant outcomes after implementation of a care process model for febrile infants. *Pediatrics* 2012;130:e16–24.
- Klinger G, Chin CN, Beyene J, *et al.* Predicting the outcome of neonatal bacterial meningitis. *Pediatrics* 2000;106:477–82.
- Kuppermann N, Dayan PS, Levine DA, *et al.* A clinical prediction rule to identify febrile infants 60 days and younger at low risk for serious bacterial infections. *JAMA Pediatr* 2019;173:342–51.
- Andreola B, Bressan S, Callegaro S, *et al.* Procalcitonin and C-reactive protein as diagnostic markers of severe bacterial infections in febrile infants and children in the emergency department. *Pediatr Infect Dis J* 2007;26:672–7.
- Gomez B, Bressan S, Mintegi S, *et al.* Diagnostic value of procalcitonin in well-appearing young febrile infants. *Pediatrics* 2012;130:815–22.
- Mintegi S, Bressan S, Gomez B, *et al.* Accuracy of a sequential approach to identify young febrile infants at low risk for invasive bacterial infection. *Emerg Med J* 2014;31:e19–24.
- England JT, Del Vecchio MT, Aronoff SC. Use of serum procalcitonin in evaluation of febrile infants: a meta-analysis of 2317 patients. *J Emerg Med* 2014;47:682–8.
- Pontrelli G, De Crescenzo F, Buzzetti R, *et al.* Accuracy of serum procalcitonin for the diagnosis of sepsis in neonates and children with systemic inflammatory syndrome: a meta-analysis. *BMC Infect Dis* 2017;17:302.
- Zahorec R. Ratio of neutrophil to lymphocyte counts—rapid and simple parameter of systemic inflammation and stress in critically ill. *Bratisl Lek Listy* 2001;102:5–14.
- de Jager CPC, van Wijk PTL, Mathoera RB, *et al.* Lymphocytopenia and neutrophil-lymphocyte count ratio predict bacteremia better than conventional infection markers in an emergency care unit. *Crit Care* 2010;14:R192.
- Lowsby R, Gomes C, Jarman I, *et al.* Neutrophil to lymphocyte count ratio as an early indicator of blood stream infection in the emergency department. *Emerg Med J* 2015;32:531–4.
- Djordjevic D, Rondovic G, Surbatovic M, *et al.* Neutrophil-To-Lymphocyte ratio, Monocyte-to-Lymphocyte ratio, platelet-to-lymphocyte ratio, and mean platelet Volume-to-Platelet count ratio as biomarkers in critically ill and injured patients: which ratio to choose to predict outcome and nature of bacteremia? *Mediators Inflamm* 2018;2018:1–15.
- Can E, Hamilcikan Şahin, Can C. The value of neutrophil to lymphocyte ratio and platelet to lymphocyte ratio for detecting early-onset neonatal sepsis. *J Pediatr Hematol Oncol* 2018;40:e229–32.
- Alkan Ozdemir S, Arun Ozer E, İlhan O, *et al.* Can neutrophil to lymphocyte ratio predict late-onset sepsis in preterm infants? *J Clin Lab Anal* 2018;32:e22338.
- Oh GH, Chung SP, Park YS, *et al.* Mean platelet volume to platelet count ratio as a promising predictor of early mortality in severe sepsis. *Shock* 2017;47:323–30.
- Shen Y, Huang X, Zhang W. Platelet-To-Lymphocyte ratio as a prognostic predictor of mortality for sepsis: interaction effect with disease severity—a retrospective study. *BMJ Open* 2019;9:e022896.
- Hoberman A, Wald ER, Reynolds EA, *et al.* Pyuria and bacteriuria in urine specimens obtained by catheter from young children with fever. *J Pediatr* 1994;124:513–9.
- Roberts KB, Subcommittee on Urinary Tract Infection, Steering Committee on Quality Improvement and Management. Urinary tract infection: clinical practice guideline for the diagnosis and management of the initial UTI in febrile infants and children 2 to 24 months. *Pediatrics* 2011;128:595–610.
- Mahajan P, Kuppermann N, Mejias A, *et al.* Association of RNA Biosignatures with bacterial infections in febrile infants aged 60 days or younger. *JAMA* 2016;316:846–57.
- Ouchenir L, Renaud C, Khan S, *et al.* The epidemiology, management, and outcomes of bacterial meningitis in infants. *Pediatrics* 2017;140. doi:10.1542/peds.2017-0476. [Epub ahead of print: 09 Jun 2017].
- Woll C, Neuman MI, Pruitt CM, *et al.* Epidemiology and etiology of invasive bacterial infection in infants  $\leq 60$  days old treated in emergency departments. *J Pediatr* 2018;200:210–7.
- Levy MM, Fink MP, Marshall JC, *et al.* 2001 SCCM/ESICM/ACCP/ATS/SIS international sepsis definitions conference. *Intensive Care Med* 2003;29:530–8.
- DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. *Biometrics* 1988;44:837–45.
- Jaskiewicz JA, McCarthy CA, Richardson AC, *et al.* Febrile infants at low risk for serious bacterial infection—an appraisal of the Rochester criteria and implications for management. Febrile Infant Collaborative Study Group. *Pediatrics* 1994;94:390–6.
- Klingenberg C, Kornelisse RF, Buonocore G, *et al.* Culture-Negative Early-Onset Neonatal Sepsis - At the Crossroad Between Efficient Sepsis Care and Antimicrobial Stewardship. *Front Pediatr* 2018;6:285.
- Oeser C, Pond M, Butcher P, *et al.* Pcr for the detection of pathogens in neonatal early onset sepsis. *PLoS One* 2020;15:e0226817.
- Stocker M, Fontana M, El Helou S, *et al.* Use of procalcitonin-guided decision-making to shorten antibiotic therapy in suspected neonatal early-onset sepsis: prospective randomized intervention trial. *Neonatology* 2010;97:165–74.
- Fjalstad JW, Stensvold HJ, Bergseng H, *et al.* Early-Onset sepsis and antibiotic exposure in term infants: a nationwide population-based study in Norway. *Pediatr Infect Dis J* 2016;35:1–6.
- Sangsoo H, Sungwoo C, Young SC. Diagnostic markers of serious bacterial infections in infants aged 29 to 90 days. *Signa Vitae* 2019;15:22–6.
- Benitz WE, Han MY, Madan A, *et al.* Serial serum C-reactive protein levels in the diagnosis of neonatal infection. *Pediatrics* 1998;102:E41.
- Hengst JM. The role of C-reactive protein in the evaluation and management of infants with suspected sepsis. *Adv Neonatal Care* 2003;3:3–13.
- Hofer N, Zacharias E, Müller W, *et al.* An update on the use of C-reactive protein in early-onset neonatal sepsis: current insights and new tasks. *Neonatology* 2012;102:25–36.
- Celik IH, Demirel G, Canpolat FE, *et al.* Inflammatory responses to hepatitis B virus vaccine in healthy term infants. *Eur J Pediatr* 2013;172:839–42.
- Korcowski B. Procalcitonin and C-reactive protein in vaccination-associated adverse reactions. *Pediatr Infect Dis J* 2004;23:283.