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# Procalcitonin and Interleukin-6 in pediatric patients admitted with suspected sepsis at Tertiary Care Hospital

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**Background** : *Bacterial sepsis remains one of the leading causes of mortality and morbidity among children. Early identification of individuals at risk of developing life-threatening sepsis could enable early treatment and improve outcomes. Procalcitonin (PCT), the peptide hormone precursor of calcitonin, and Interleukin-6 (IL-6), the pro-inflammatory cytokine, are both substances that present in higher concentrations after inflammation. The potential value of measuring PCT and IL-6 levels are recognized as biological markers in pediatric patients with systemic inflammatory response syndrome (SIRS) in both early diagnosis of sepsis and to differentiate sepsis from the other SIRS non-septic conditions, has higher accuracy than the routine laboratory tests pragmatically such as total leukocytes count and C-reactive protein (CRP).*

**Objectives** : *To define the clinical and laboratory predictive factors for early diagnosis in pediatric bacterial sepsis and distinguishing from the other SIRS non-septic conditions, including the development of the risk scoring system for pediatric bacterial sepsis prediction.*

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**Methods** : *The diagnostic prediction research was designed from the prospective data from September 2015 to June 2016 at the Department of Pediatrics, Bhumibol Adulyadej Hospital, Bangkok, Thailand. Forty-nine patients from the age of 1 month to 15 years old who met the criteria of SIRS definition were recruited into the study. Multivariable logistic regression was performed to select the strongest predictors then transformed to develop the final bacterial sepsis score (FBAC score).*

**Results** : *The best clinical and laboratory predictors included female gender, PCT, IL-6, CRP levels prior to treatment on admission date and CRP level at 24 hours after starting of the treatment. The developed FBAC score predicted bacterial sepsis correctly with an AuROC of 86.5%. The patients who have FBAC scores below 10 (low risk category) will have the chance to be bacterial sepsis 10 times less (likelihood ratio of positive; LHR + 0.1, P - value 0.01) and the potential bacterial septic patients will have more than 6 times increasingly (LHR+ 6.13, P - value 0.02) if their total FBAC scores are higher than 30 (high risk category).*

**Conclusions** : *The developed FBAC scores will help pediatricians to predict bacterial sepsis for early treatment intervention and can distinguish bacterial sepsis from the other SIRS non-septic conditions.*

**Keywords** : *Procalcitonin, Interleukin-6, systemic inflammatory response syndrome, bacterial sepsis, final bacterial sepsis score.*

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**พาณิภัค เต็มบุญนาค, กิตติพล คล้ายคลึง, วิศรุต การุณบุญญานันท์, ปัญจฉัตร รัตนมงคล. โปรแคลซิโตนิน และอินเตอร์ลิวคิน 6 ในผู้ป่วยเด็กที่สงสัยภาวะติดเชื้อแบคทีเรียในกระแสเลือด. จุฬาลงกรณ์เวชสาร 2561 ม.ค. – ก.พ.;62(1): 9 – 25**

**เหตุผลของการทำวิจัย** : *ภาวะติดเชื้อแบคทีเรียในกระแสเลือดเป็นสาเหตุสำคัญของภาวะทุพพลภาพ และการเสียชีวิตในผู้ป่วยเด็ก ซึ่งถ้าผู้ป่วยได้รับการวินิจฉัยและการรักษาอย่างรวดเร็วและเหมาะสมจะทำให้เกิดผลการรักษาที่มีประสิทธิภาพมากขึ้น ปัจจุบันการวินิจฉัยภาวะติดเชื้อแบคทีเรียในกระแสเลือด อาศัยการติดตามอาการทางคลินิกของผู้ป่วยที่เข้าได้และการเพาะเชื้อจากเลือด ซึ่งต้องใช้เวลาหลายวัน ดังนั้นการทำนายภาวะติดเชื้อแบคทีเรียในกระแสเลือดตั้งแต่ระยะเริ่มต้นก่อนการรักษาจึงมีความสำคัญเป็นอย่างยิ่ง โปรแคลซิโตนิน (procalcitonin) และอินเตอร์ลิวคิน 6 (Interleukin-6) เป็นสารที่ถูกหลั่งออกมาเมื่อร่างกายมีอาการตอบสนองต่อการอักเสบทั่วร่างกาย (systemic inflammatory response syndrome, SIRS) จึงสามารถช่วยในการวินิจฉัยภาวะติดเชื้อในกระแสเลือด และแยกความแตกต่างระหว่างผู้ป่วยเด็กที่มีอาการแสดงของภาวะ SIRS ที่เกิดจากการติดเชื้อแบคทีเรียในกระแสเลือดออกจากภาวะ SIRS ที่เกิดจากสาเหตุอื่นได้อย่างรวดเร็วและแม่นยำกว่าค่าทางห้องปฏิบัติการพื้นฐานที่ใช้ในปัจจุบัน เช่น ซีรีแอคทีฟโปรตีน (C-reactive protein) เป็นต้น*

**วัตถุประสงค์** : *เพื่อศึกษาอาการ อาการแสดงทางคลินิกและผลทางห้องปฏิบัติการที่ช่วยในการวินิจฉัยภาวะติดเชื้อแบคทีเรียในกระแสเลือดของผู้ป่วยเด็ก รวมทั้งศึกษาปัจจัยที่ช่วยแยกผู้ป่วยเด็กที่มีอาการแสดงของภาวะ SIRS ที่เกิดจากการติดเชื้อแบคทีเรียในกระแสเลือดออกจากภาวะอื่น ๆ*

**วิธีการทำวิจัย** : *เป็นการศึกษาเชิงวินิจฉัยและคาดคะเน (diagnostic prediction research) โดยเก็บข้อมูลไปข้างหน้า ระหว่างวันที่ 1 กันยายน 2558 ถึง 30 มิถุนายน 2559 ที่หอผู้ป่วยกุมารเวชกรรม และหออภิบาลผู้ป่วยกุมารเวชกรรม โรงพยาบาลภูมิพลอดุลยเดช กรมแพทย์ทหารอากาศ ในผู้ป่วยเด็กจำนวน 50 คน ซึ่งอายุระหว่าง 1 เดือนถึง 15 ปี ที่มีอาการอาการแสดงทางคลินิกและผลทางห้องปฏิบัติการเข้าได้กับภาวะ SIRS โดยได้ส่งตรวจระดับโปรแคลซิโตนิน ระดับอินเตอร์ลิวคิน 6 และระดับซีรีแอคทีฟโปรตีนก่อนทำการรักษา หลังเริ่มการรักษา 24 ชั่วโมงและหลังเริ่มการรักษา 72 ชั่วโมงตามลำดับ แล้วนำข้อมูลทั้งหมดมาวิเคราะห์โดยวิธี multivariate logistic regression เพื่อหาปัจจัยทำนายที่มีความน่าเชื่อถือมากที่สุด (best predictive factors) แล้วนำมาพัฒนาต่อเป็น final bacterial sepsis score (FBAC score) เพื่อใช้ทำนายโอกาสเป็นภาวะติดเชื้อแบคทีเรียในกระแสเลือด*

**ผลการศึกษา** : *ข้อมูลอาการ อาการแสดงทางคลินิกและผลทางห้องปฏิบัติการที่มีความน่าเชื่อถือในการทำนายภาวะติดเชื้อแบคทีเรียในกระแสเลือดของผู้ป่วยเด็กมากที่สุด คือ เพศหญิง ระดับโปรแคลซิโตนินก่อนทำการรักษา ระดับอินเตอร์ลิวคิน 6 ก่อนทำการรักษา ระดับซีรีแอคทีฟโปรตีนก่อนทำการรักษา และระดับซีรีแอคทีฟโปรตีนหลังเริ่มการรักษา 24 ชั่วโมง (P - value < 0.1 และ AuROC > 0.7) FBAC score มีค่า Area Under an ROC Curve (AuROC) เท่ากับร้อยละ 86.5 ค่า positive predictive value เท่ากับ ร้อยละ 71.4 และค่า negative predictive value เท่ากับร้อยละ 79.1 โดยผู้ป่วยที่มี FBAC score น้อยกว่าหรือเท่ากับ 10 คะแนน (low risk category) มีโอกาสเป็นภาวะติดเชื้อแบคทีเรียในกระแสเลือดลดลง 10 เท่า (likelihood ratio of positive; LHR + 0.1, P - value 0.01) แต่ถ้ามี FBAC score มากกว่าหรือเท่ากับ 30 คะแนน (high risk category) มีโอกาสเป็นการติดเชื้อแบคทีเรียในกระแสเลือดเพิ่มขึ้น 6 เท่า (likelihood ratio of positive; LHR + 6.13, P - value 0.02)*

**สรุป** : *จากงานวิจัยพบว่า FBAC score สามารถเป็นเครื่องมือในการทำนายภาวะติดเชื้อแบคทีเรียในกระแสเลือดของผู้ป่วยเด็กได้ ซึ่งอาจช่วยให้กุมารแพทย์สามารถวินิจฉัยได้รวดเร็ว และสามารถให้การรักษาตั้งแต่ระยะแรกได้อย่างมีประสิทธิภาพมากขึ้น นอกจากนั้นสามารถใช้แยกความแตกต่างระหว่างผู้ป่วยเด็กที่มีอาการแสดงของภาวะ SIRS ที่เกิดจากการติดเชื้อแบคทีเรียในกระแสเลือด ออกจากภาวะ SIRS ที่เกิดจากสาเหตุอื่นได้อย่างรวดเร็วและแม่นยำมากยิ่งขึ้น*

**คำสำคัญ** : *โปรแคลซิโตนิน, อินเตอร์ลิวคิน 6, กลุ่มอาการตอบสนองต่อการอักเสบทั่วร่างกาย, การติดเชื้อแบคทีเรียในกระแสเลือด, final bacterial sepsis score.*

Bacterial sepsis is one of the most common causes of mortality and morbidity among children especially in which severe sepsis with organ failure and septic shock which particularly profound circulatory, cellular and metabolic abnormalities associated with greater risk of mortality than with sepsis alone. Prompt and proper empirical antibiotic treatment is important for septic management. Blood culture is known as the gold standard test for diagnosis sepsis. However, its result hardly shows within 24 hours. [1] Total blood leukocytes count and C-reactive protein (CRP) level are universally used to be routine investigations for diagnosing sepsis but those still have limitations in accuracy and sometimes mislead the results.[2]

Procalcitonin (PCT) is the peptide precursor of calcitonin, a hormone that is synthesized by the parafollicular C cells of the thyroid gland and involved in calcium homeostasis. Normal serum PCT level is very low (<0.1 ng/ml).[3] PCT levels are obviously higher both in patients with sepsis and those with systemic inflammatory response syndrome (SIRS) from infection than the normal population.[4,5] Significantly, many studies have shown PCT is a biological marker for early diagnosis and as an indicator of severity among children with sepsis.[6,7] PCT is proved to be superior to CRP for diagnosis sepsis. [8 -11] After inflammation, PCT level rises at 4-hour interval (6 hours in CRP), peaking around 8 hours (36 - 50 hours in CRP) and takes 48 hours to reach normal level after appropriate treatment (72 - 96 hours in CRP). Furthermore, PCT also has more sensitivity and specificity for diagnosis sepsis, 92.6% and 97.5%, respectively.[12]

Interleukin-6 (IL-6) is an interleukin which acts as both pro-inflammatory cytokines and anti-inflammatory myokines, is secreted by T-cells and macrophages to stimulate immune response, e.g. during infection and after trauma, especially burns or other tissue damage leading to inflammation.[13, 14] Normally, IL-6 level is detected less than 5 pg/ml in the normal population but the level can rise within an hour after getting inflammation.[15] IL-6 is an early predictor for mortality in newly admitted patients who come with clinically SIRS. [16] IL-6 levels in septic shock patients are obviously higher than in sepsis patients. [17 - 20] In comparison with PCT and IL-6 levels are extremely higher at zero and twelve hours in septic patients with septic shock.[21] Moreover, some studies have shown PCT level is higher specificity whereas IL-6 is more sensitive and valuable for distinguishing bacteremia among oncohematologic patients with febrile neutropenia. [22]

According to neonatal sepsis has the unique complexity [23, 24], therefore the neonatal group is excluded from this study. Our hypothesis is that PCT and IL-6 levels are useful for early diagnosis of bacterial sepsis among children after neonatal period.

**Objectives**

The aim of our study is to define the clinical and laboratory predictive factors for early diagnosis in pediatric bacterial sepsis and distinguish sepsis from other SIRS non-sepsis, including the development of a scoring system for the prediction of pediatric bacterial sepsis.

## Patients and Methods

### *Study design and participants*

The diagnostic prediction research was designed from prospective data at the Pediatrics Department of Bhumibol Adulyadej Hospital, Royal Thai Air Force, Bangkok, Thailand during a 10-month period from 1 September 2015 to 30 June 2016.

The study has been approved by our Medical Research Institute and the Ethics Committee. After obtaining the informed consent, the pediatric patients between the age of 1 month and 15 years old who met the criteria of systemic inflammatory response syndrome (SIRS) definition were recruited into the study.

### *Diagnosis of SIRS and sepsis*

According to the Pediatric Sepsis Consensus Congress 2015, systemic inflammatory response syndrome (SIRS) is defined as a patient having at least two of the following four criteria: core temperature of > 38.5 $^{o}$C or < 36$^{o}$C, tachycardia (defined as mean heart rate > 2 SD above normal with the absence of external stimulus, chronic drugs, or painful stimuli, or otherwise unexplained persistent elevation over 0.5 - 4 hours) or for children < 1 year : bradycardia (defined as mean heart rate < 10$^{th}$ percentile for age in the absence of external vagal stimulus, beta-blockers, congenital heart diseases, or otherwise unexplained persistent depression over 30 minutes), mean respiratory rate > 2 SD above normal of age or mechanical ventilator for an acute process not related to underlying neuromuscular disease or the receipt of general anesthesia and elevated or depressed total leukocyte counts for age (not secondary to chemotherapy-induced leukopenia) or > 10% immature neutrophils. One of these must be abnormal temperature or leukocyte count. [25]

Sepsis is defined when 2 or more SIRS criteria are present with a culture-proven or clinically identified infection. [25, 26] In our study, we used clinical follow-up data at the final course of treatment and /or positive hemoculture as the reference standard.

Patients with small cell lung carcinoma, medullary carcinoma of the thyroid gland, major trauma, cardiogenic shock and severe burn were excluded from this study.

### *Data collection and laboratory measurement*

The clinical data of all recruited patients was collected including gender, age, underlying diseases, duration of fever, body temperature, blood pressure and heart rate.

The first blood sample was taken on admission date were complete blood count (CBC), blood urea nitrogen (BUN), creatinine, electrolytes, liver function test, blood culture, CRP (CRP-1), PCT (PCT-1), and IL-6 (IL-6-1). Subsequent two samples of CRP, PCT and IL-6 were taken at 24 hours (CRP-2, PCT-2, IL-6-2) and 72 hours later (CRP-3, PCT-3, IL-6-3). Resulting times of CRP, PCT, IL-6 were around 1 hour.

Blood volume at least 1 - 3 ml was taken for culture of all recruited patients before antibiotic was given. Blood culture was collected into BacT/Alert PF Plus Aerobic Pediatric bottle, delivered to the laboratory room not exceeding 1 hour, then incubated in Virtuo BacT/Alert and delivered into 5.0% blood agar plate and Macconkey agar plate.

CRP levels were measured by Roche P800 laboratory kit test, procalcitonin levels were measured by used Roche E170 laboratory kit test and IL-6 levels

were measured by Roche E601.

A value of PCT 0.5 to 2 ng/ml meant possible infection, 2 to 10 ng/ml indicated most likely sepsis and a value above 10 ng/ml showed severe bacterial sepsis or septic shock while IL-6 a value of IL-6 > 131 pg/ml referred to bacterial sepsis. [27, 28] A plasma CRP of 50 mg/L or more was highly suggestive of sepsis.[29]

***Statistical analysis***

Clinical and laboratory data, as the potential predictors of sepsis obtained at time of admission included age, gender, underlying disease, onset of fever, body temperature, heart rate, shock status, total leukocytes count, absolute neutrophils count, band form neutrophils count, serum creatinine, serum total bilirubin, serum albumin, serum alanine aminotransferase (ALT), serum bicarbonate, serum CRP, serum PCT, serum IL-6, and hemoculture. Additionally, serum CRP, serum PCT and serum IL-6 were repeated at 24 and 72 hours later after starting treatment.

Two groups of patients, finally diagnosed from clinical follow-up data as sepsis and non-sepsis groups, were compared for evidence of differences (*P* - value) in all clinical and laboratory data with *t* - test, rank sum tests, chi-square or exact probability test as appropriate. Prediction by each clinical and laboratory data was calculated by univariate logistic regression analysis and presented as an area under the receiver operating characteristic (AuROC) curve. Strong (AuROC > 0.7) and significant (*P* – value < 0.1) clinical and laboratory data were categorized to facilitate odd ratio calculation, under multivariable logistic regression. Discriminative performance of the model was calculated by an AuROC curve. Regression coefficients of each level of each predictor were divided by the smallest coefficient of the model to transform into an item risk score. Scores for each predictors were added up to obtain a total risk score, name final bacterial sepsis score (FBAC score). Score prediction of sepsis was done by using this FBAC score as the only summary predictor in the logistic model. Discrimination of the scores was presented with an AuROC curve. Calibration of the prediction was analyzed with Hosmer-Lemeshow statistics. Score predicting risks and observed risk were compared and presented in a graph as a risk curve. Risk scores were categorized into three risk levels, low, moderate and high. Predictive ability of each risk score level was calculated and presented as a likelihood ratio of positive (LHR+), 95.0% confidence interval and its significant level (*P* - value). Model and score performance are also expressed as sensitivity, specificity, predictive values and overall accuracy. All *P* - values < 0.05 were regarded as significant. Statistical analyses were done using STATA version 14.

It was estimated that a total sample of at least 40 patients would be adequate in our study. We chose the maximum sample size, calculated by two independent mean difference formula, by comparing all predictors among sepsis and non-sepsis SIRS patients, with a two tailed alpha level of 0.05 and a power of 90.0%, according to the 2 studies. [30, 31]

**Results**

A total of fifty patients, diagnosed with SIRS were included in the study. Twenty patients (40.0%) had final diagnosis with bacterial sepsis. The

univariate logistic analysis of all clinical characteristics and laboratory data are shown in Table 1 and 2. There were significantly female patients in sepsis group more than non–sepsis group (65.0% *vs* 35.0%, *P* - value 0.003). Other clinical characteristics as the age, underlying diseases of patients, duration of fever, shock status, body temperature, heart rate were not statistically different among sepsis and non-sepsis group. The laboratory data as total leukocytes count, absolute neutrophils count, band form neutrophils count, serum creatinine, serum bicarbonate, serum albumin, and serum alanine aminotransferase (ALT) were also not statistically different between the two groups.

Mean levels of all biomarkers on admission date prior to treatment, 24-hour interval and 72-hour interval after treatment between sepsis and non-sepsis groups are shown in Table 3. In comparison, there were significant differences of PCT-1, PCT-2, PCT-3, IL-6-1, IL-6-2, IL-6-3, CRP-1 and CRP-2 levels between sepsis and non-sepsis groups (*P* - value < 0.1) and AuROC > 0.7. Among all clinical and laboratory predictors, the prediction ability as measured by the AuROC curve was highest for IL-6-1.

After the univariate logistic analysis, female gender, PCT-1, PCT-2, PCT-3, IL-6-1, IL-6-2, IL-6-3, CRP-1 and CRP-2 were chosen as the potential predictive factors due to *P* - value < 0.1 and AuROC > 0.7. The values of PCT-1, PCT-2, PCT-3, IL-6-1, IL-6-2, IL-6-3, CRP-1 and CRP-2 levels were categorized into 2 groups according to reference cut-off levels as shown in Table 4 to facilitate odd ratio calculation. The best predictors were then selected to performed multivariable logistic regression analysis.

**Table 1.** Clinical characteristics of sepsis cases vs. non sepsis cases, evidence of difference. (*P* - value) and area under the curve (AuROC)

| **Characteristics** | **Sepsis (n = 20)** | **Non sepsis (n = 30)** | ***P*** | **AuROC** |
|---|---|---|---|---|
| Age (months); mean (95%CI) | 59.4 (32.6 - 86.2) | 57.3 (33.9 - 80.8) | 0.91 | 0.51 |
| Gender (female/male); n (%) | 13 (65.0%) / 7(35.0%) | 6 (20.0%) / 24 (80.0%) | 0.01 | 0.73 |
| Underlying disease; n (%) | | | | |
| - Yes | 7 (35.0%) | 5 (16.7%) | 0.37 | 0.60 |
| - No | 13 (65.0%) | 25 (83.3%) | | |
| Duration of fever(h); mean (95%CI) | 64.6 (38.1 - 91.0) | 71.7 (44.1 - 99.2) | 0.71 | 0.50 |
| Shock; n (%) | 9 (45.0%) | 5 (16.7%) | 0.06 | 0.64 |
| Body temp ($^{o}$c); mean (95%CI) | 38.5 (38.0 - 39.0) | 38.4 (38.1 - 38.8) | 0.86 | 0.55 |
| Heart rate; mean (95%CI) | 145 (134 - 158) | 136 (127 - 146) | 0.21 | 0.56 |

**Table 2.** Laboratory data on admission date of sepsis cases vs non sepsis cases, evidence of difference (*P* - value) and area under the curve (AuROC).

| Laboratory data | Sepsis (n = 20) Mean (95%CI) | Non-sepsis (n = 30) Mean (95%CI) | *P* | AuROC |
|---|---|---|---|---|
| WBC count (cell/cumm$^3$) | 12,760.5 (6,361.6 - 19,159.4) | 16,400.7 (12,565.7 - 20,235.6) | 0.29 | 0.35 |
| ANC (cell/cumm$^3$) | 11,538.5 (4,296.1 - 18,780.9) | 11,514.2 (7,915.8 - 15,112.5) | 0.99 | 0.41 |
| Band form neutrophils (%) | 6.5 (3.0 - 10) | 7.5 (4.4 - 10.6) | 0.70 | 0.48 |
| Creatinine (mg/dL) | 0.4 (0.3 - 0.5) | 0.4 (0.3 - 0.5) | 0.92 | 0.52 |
| Total bilirubin (mg/dL) | 0.8 (0.3 - 1.3) | 0.4 (0.3 - 0.4) | 0.05 | 0.60 |
| Serum albumin (g/dL) | 3.1 (2.8 - 3.4) | 4.6 (3.3 - 5.9) | 0.06 | 0.12 |
| AST (U/L) | 58.0 (28.4 - 87.6) | 34.0 (25.6 - 42.5) | 0.07 | 0.61 |
| ALT (U/L) | 39.2 (20.4 - 57.9) | 40.3 (23.6 - 57.0) | 0.92 | 0.51 |
| Serum bicarbonate (mEq/L) | 17.2 (12.6 - 18.8) | 23.2 (15.2 - 31.1) | 0.21 | 0.45 |

WBC White blood cell, ANC Absolute neutrophil count, AST Aspartate aminotransferase , ALT Alanine- aminotransferase

**Table 3.** Laboratory data on admission date, 24 hours and 72 hours after admission of case sepsis and non sepsis, evidence of difference (*P* - value) and area under the curve (AuROC)

| Laboratory data | Sepsis (n = 20) Mean (95%CI) | Non-sepsis (n = 30) Mean (95%CI) | *P* | AuROC |
|---|---|---|---|---|
| PCT-1 (ng/mL) | 21.1 (7.8 - 34.5) | 3.4 (1.6 - 5.2) | 0.01 | 0.75 |
| PCT-2 (ng/mL) | 20.4 (4.0 - 36.71) | 6.0 (1.5 - 13.5) | 0.07 | 0.77 |
| PCT-3 (ng/mL) | 13.4 (-3.2 - 29.9) | 1.4 (0.1 - 2.7) | 0.07 | 0.70 |
| IL-6-1 (pg/mL) | 682.8 (82.5 - 1,283.1) | 49.7 (26.2 - 73.1) | 0.01 | 0.86 |
| IL-6-2 (pg/mL) | 193.0 (0.3 - 1.3) | 34.1 (6.9 - 379.0) | 0.03 | 0.83 |
| IL-6-3 (pg/mL) | 86.3 (1.1 - 171.5) | 22.6 (0.8 - 46.0) | 0.08 | 0.77 |
| CRP-1 (mg/dL) | 127.5 (73.8 - 181.3) | 58.8 (28.6 - 89.0) | 0.01 | 0.71 |
| CRP-2 (mg/dL) | 127.6 (64.4 - 178.8) | 43.3 (25.8 - 60.9) | 0.01 | 0.80 |
| CRP-3 (mg/dL) | 43.7 (16.4 - 70.9) | 29.6 (10.2 - 49.0) | 0.37 | 0.87 |

PCT-1; PCT on admission date, PCT-2; PCT at 24 hours later, PCT-3; PCT at 72 hours later , IL-6-1; IL-6 on admission date, IL-6 -2; IL-6 at 24 hours later, IL-6-3; IL-6 at 72 hours later, CRP-1; CRP on admission date, CRP-2; CRP at 24 hours later, CRP-3; CRP at 72 hours later

**Table 4.** Categorized laboratory data into groups according to the reference cut-off levels. [29]

| Laboratory data | Sepsis | Non sepsis | *P* | AuROC |
|---|---|---|---|---|
| PCT-1 (ng/ml) | | | | |
| < 2 | 6 (30.0%) | 20 (66.7%) | 0.02 | 0.68 |
| ≥ 2 | 14 (70.0%) | 10 (33.3%) | | |
| PCT-2 (ng/ml) | | | | |
| < 2 | 6 (37.5%) | 16 (66.7%) | 0.11 | 0.65 |
| ≥ 2 | 10 (62.5%) | 8 (33.3%) | | |
| PCT-3 (ng/ml) | | | | |
| < 2 | 8 (57.1%) | 16 (80.0%) | 0.25 | 0.61 |
| ≥ 2 | 6 (42.9%) | 4 (20.0%) | | |
| IL-6-1 (pg/ml) | | | | |
| < 131 | 10 (50.0%) | 26 (89.7%) | 0.01 | 0.70 |
| ≥ 131 | 10 (50.0%) | 3 (10.3%) | | |
| IL-6-2 (pg/ml) | | | | |
| < 131 | 10 (66.7%) | 21 (87.5%) | 0.22 | 0.60 |
| ≥131 | 5 (33.3%) | 3 (12.5%) | | |
| IL-6-3 (pg/ml) | | | | |
| < 131 | 12 (85.7%) | 19 (95.0%) | 0.56 | 0.56 |
| ≥ 131 | 2 (14.3%) | 1 (95.0%) | | |
| CRP-1 (mg/dl) | | | | |
| < 50 | 6 (31.6%) | 19 (63.3%) | 0.04 | 0.66 |
| ≥ 50 | 13 (68.4%) | 11 (36.7%) | | |
| CRP-2 (mg/dl) | | | | |
| < 50 | 2 (13.3%) | 15 (62.5%) | 0.01 | 0.75 |
| ≥ 50 | 13 (86.7%) | 9 (37.5%) | | |

After data categorization, the best 5 potential predictors remained female gender, PCT-1, IL-6-1, CRP-1 and CRP-2. They were chosen to make an exploratory model. An items score was assigned to each level of the 5 clinical and laboratory data by simple transformation of its logistic regression coefficient as shown in Table 5. The final bacterial sepsis score (FBAC score) was obtained by adding up the item scores.

The discriminative ability of the FBAC score which ranges from 0 to 37, could be observed by the different percentage distribution between sepsis and non-sepsis groups as shown in Figure 1. The lower FBAC scores were more likely to be non-sepsis. On the other hand, the higher FBAC score were more likely to be sepsis. The FBAC score predicted bacterial sepsis with an AuROC of 86.5% as shown in Figure 2 and with the Hosmer-Lemeshow goodness of fit test of 0.55. In the risk curve showed that the scores predicted the risk of sepsis move upward when FBAC score increase, with close calibration to the actual or observed risks as shown in Figure 3.

**Table 5.** Best multivariable clinical predictors, odds ratio (OR), 95% confidence interval (CI), evidence of difference (*P* - value) and assigned item scores.

| Predictors | OR | 95% CI | *P* | Score |
|---|---|---|---|---|
| Gender | | | | |
| Male | 1.00 | reference | - | 0 |
| Female | 13.32 | 1.61 - 109.8 | 0.01 | 9.5 |
| PCT on admission date (ng/ml) | | | | |
| < 2 | 1.00 | reference | - | 0 |
| ≥ 2 | 1.31 | 0.05 - 10.0 | 0.83 | 1 |
| IL-6 on admission date (pg/ml) | | | | |
| < 131 | 1.00 | reference | - | 0 |
| ≥ 131 | 5.51 | 0.43 - 70.3 | 0.19 | 6 |
| CRP on admission date (mg/dl) | | | | |
| < 50 | 1.00 | reference | - | 0 |
| ≥ 50 | 6.46 | 0.01 - 3.0 | 0.22 | 7 |
| CRP at 48 hours later (mg/dl) | | | | |
| < 50 | 1.00 | reference | - | 0 |
| ≥ 50 | 38.78 | 1.35 - 1,107.0 | 0.03 | 13.5 |

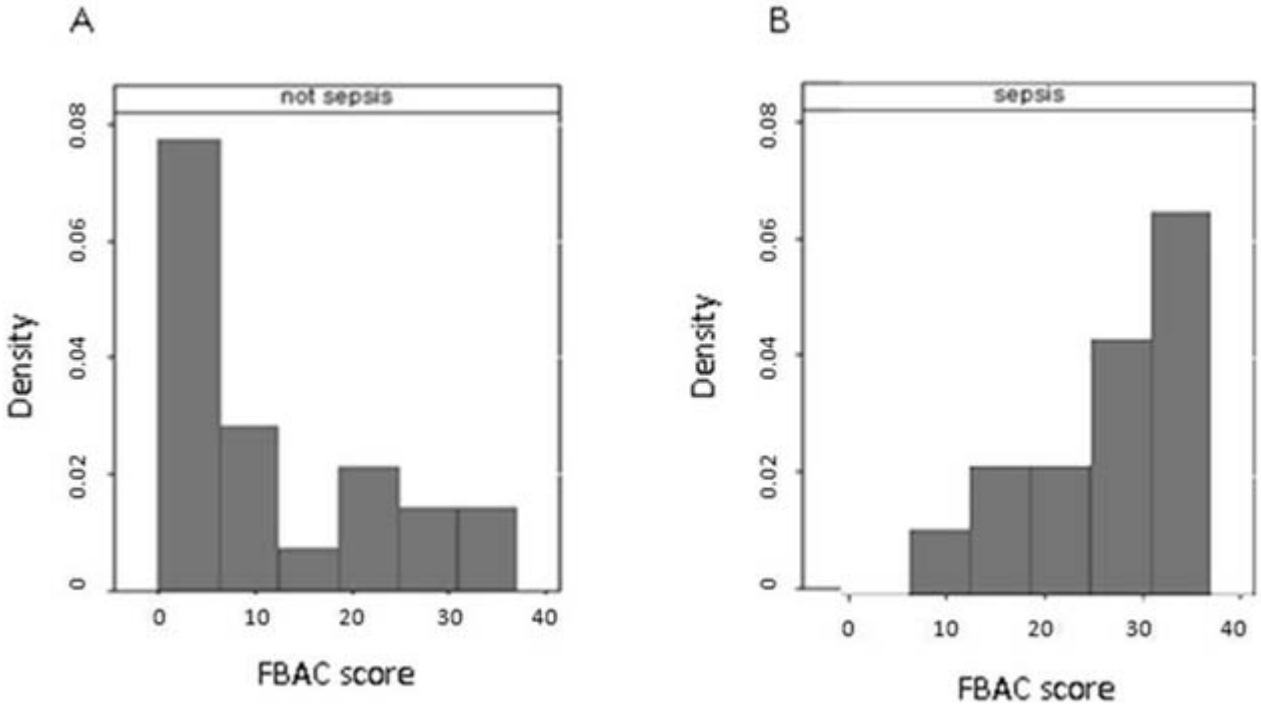


**Figure 1.** Percentage distribution of final bacterial sepsis (FBAC) score of A (not sepsis) and B (sepsis).

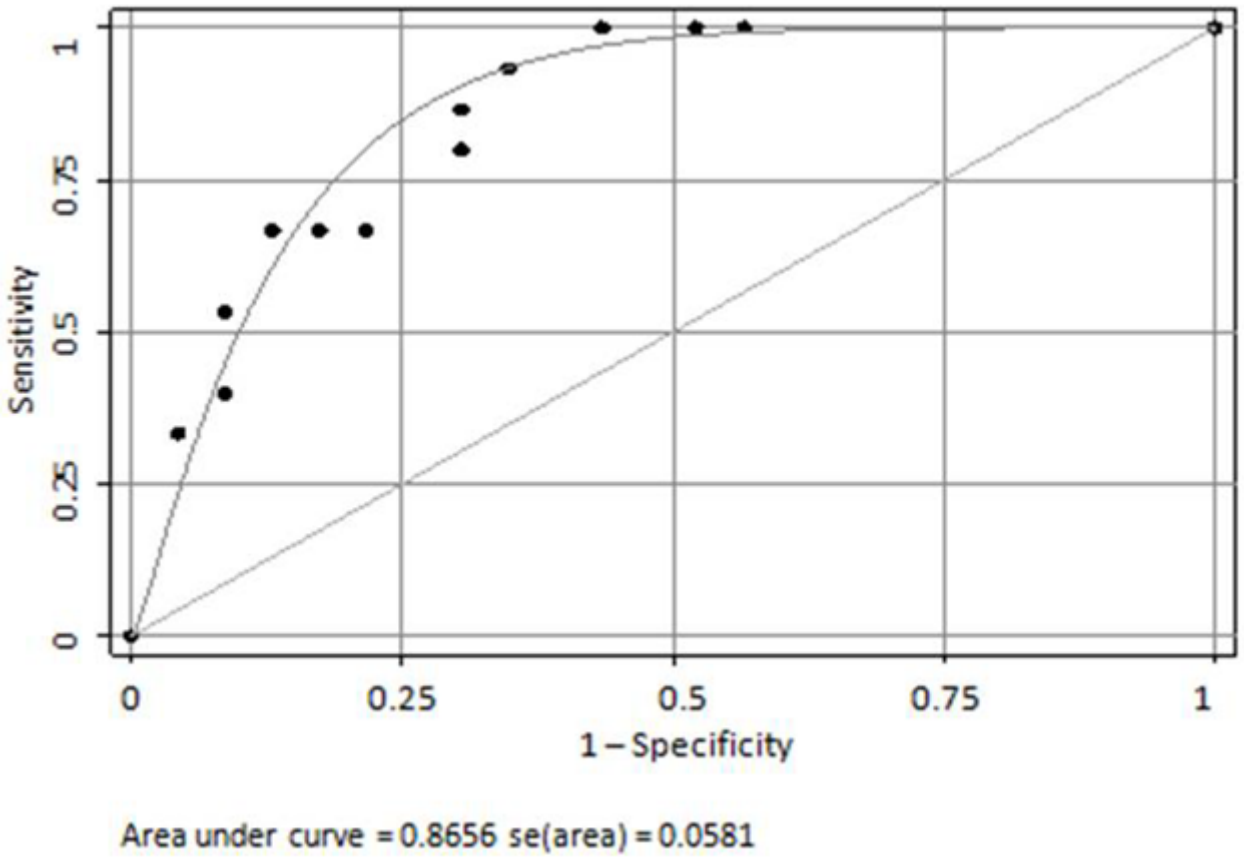


**Figure 2.** Area under receiver operating characteristic curve of FBAC scores on prediction of bacterial sepsis.

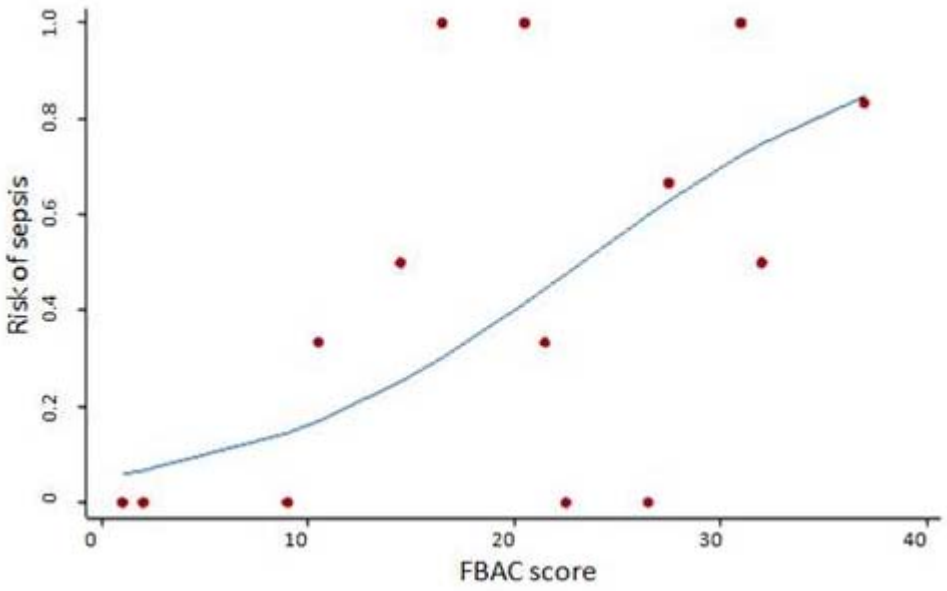


**Figure 3.** Observed risk (dot) vs. scores predicted risk (solid line) of bacterial sepsis.

The FBAC score were categorized into 3 risk groups, low (below 10) when the slope of the risk curve was lowest, moderate (10 to 30), and high (above 30) when the slope was highest to facilitate interpretation as shown in Table 5. At low category of FBAC score (below 10), the SIRS patients were significantly lower probability to be sepsis (LHR+ 0.10 and *P* - value 0.01) and at high category of FBAC score (above 30), the SIRS patients were significantly higher probability to be sepsis (LHR+ 6.13 and *P* - value 0.02).

The accuracy of FBAC score in distinguishing SIRS non-septic patients from septic patients is shown in Table 7. The score yielded a sensitivity of 66.67%, positive predictive value of 71.43%, of importance the value reached to 82.61% and 79.17% in specificity and negative predictive value, respectively.

In this study, Only one patient had positive hemoculture. He was a boy, 7 months of age. His underlying disease were cerebral palsy and epilepsy. He was diagnosed as pneumonia with septic shock. His intial PCT, IL-6 and CRP were 5.8, 319.5 and 66.9 respectively. He received Piperacillin/Tazobactam. At 24 hour, his PCT, IL-6 and CRP became 2.8, 156.7 and 82.7 respectively. He died before 72 hours. His sputum culture was Stenotrophomonas maltophilia (MDR). The hemoculture, showed Pseudomonas aeruginosa (Extremly drug resisitant). The results of cultures returned after 3 days.

**Table 6.** Distribution of sepsis vs. non sepsis into low, moderate and high probability categories, likelihood ratio of positive (LHR+) and 95% confidence interval (CI).

| Probability categories | FBAC score | Sepsis (n = 15)* | | Non sepsis (n = 23)* | | LHR+ | 95% CI | *P*-value |
|---|---|---|---|---|---|---|---|---|
| | | n | % | n | % | | | |
| Low | < 10 | 1 | (6.7) | 15 | (65.2) | 0.10 | 0.1 - 0.8 | 0.01 |
| Moderate | 10 - 30 | 6 | (40.0) | 6 | (26.1) | 1.53 | 0.3 - 6.9 | 0.52 |
| High | > 30 | 8 | (53.3) | 2 | (8.7) | 6.13 | 1.0 - 64.6 | 0.02 |

LHR+: likelihood ratio of positive

*number of cases: with complete data

**Table 7.** Sensitivity and specificity of final bacterial sepsis (FBAC) score.

| | Final bacterial sepsis (FBAC) score |
|---|---|
| Sensitivity (%) | 66.67% |
| Specificity (%) | 82.61% |
| Positive predictive value (%) | 71.43% |
| Negative predictive value (%) | 79.17% |
| Accuracy (%) | 76.31% |

## Discussion

The 50 pediatric patients who have been diagnosed with SIRS over 10-months period, there were 31 male (62.0%) and 19 female patients (38.0%) and male to female ratio is 1.63: 1 that are similar to other studies in other countries.[32]

In the present studies show during the infectious process and sepsis, the effects of testosterone and androgen hormones can suppress the cell-mediated immune (CMI) response (immune depression). On the other hand, female hormones such as estrogen can promote the CMI response (immune modulation) that make the male is more likely to infect more often than female.[32] In our study, comparison between sepsis and SIRS non-sepsis groups, there are 13 female patients from 20 patients (65.0%) in sepsis group while there are 24 male patients from 30 patients in SIRS non-sepsis group (80.0%) this was distinguished from the past studies. Because this study was done with patients under the age of 15 years that is not under the reproductive age group, which has low level of estrogen; thus, there is no significant difference between males and females and no effect on female hormone levels to stimulate the CMI response.

Changes of leukocyte count, a routine laboratory test for sepsis, are common among septic patients. In fact, white blood cells > 12,000/cu.mm. or < 4,000/cu.mm. or shift to the left > 10.0% of immature neutrophils (bands) are one of the four SIRS criteria employed to diagnosis of sepsis but normal white blood cell count does not exclude sepsis, because many patients with sepsis have no leukocytosis, and many patients with leukocytosis have no infection. The white blood cell (WBC) count is a not suitable test to determine infection and should be used only as part of validated multivariable decision rules for medical decision making. [33]

Prediction of bacterial sepsis has been a challenge of practice in pediatrics. It remains one of the leading causes of mortality and morbidity among children. Early identification of individuals at risk of developing life-threatening sepsis could enable early treatment and improve outcomes. In our study, the strongest potential clinical and laboratory predictors of sepsis are female gender, PCT concentration prior to treatment more than 2 ng/ml, IL-6 prior to the treatment more than 131 pg/ml, CRP prior to treatment and CRP at 24 hours after treatment more than 50 mg/dl. All these predictors can be assessed and the results obtained within one hour, therefore they can help pediatricians to predict probability of bacterial sepsis at the early course of treatment without waiting for blood culture or more blood sampling to determine.[34, 35]

Moreover, we have also developed simple final bacterial sepsis (FBAC) score. In our setting, the patients who scored below 10, will have a condition sepsis decreased 10 time (LHR + 0.1) and potential sepsis patients were more than six times the rate of total FBAC score more than 30 (LHR+ 6.13). The FBAC scores will be very useful for pediatrician to predict bacterial sepsis and differentiate between septic and SIRS non-septic conditions to make decision on early antibiotic treatment by using this score as the guideline.

## Conclusion

The predictors of bacterial sepsis in SIRS patients from our study are female gender, high levels

of biomarker as PCT, IL-6 and CRP, the result of which could be obtained early in the course of treatment. A developed simple FBAC scores from all of the predictors will enable pediatricians to predict bacterial sepsis for early treatment intervention and can distinguish bacterial sepsis from the other SIRS non-septic conditions.

Further studies should be conducted in larger numbers of pediatric patients and performed in multi-centers to provide generalizability of the predictive model.

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