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ORIGINAL ARTICLE

Procalcitonin for Differentiating Gram-Negative From Gram-Positive Sepsis in Children: A Retrospective Diagnostic Accuracy Study

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ABSTRACT

Background: Pediatric sepsis is a leading cause of morbidity and mortality, and early appropriate antibiotic treatment is crucial in improving outcomes. Procalcitonin is a biomarker that increases in bacterial infections. Some adult studies have reported higher levels in Gram-negative compared to Gram-positive sepsis, suggesting a potential role in pathogen differentiation; however, findings have been inconsistent.

Objectives: To determine the diagnostic accuracy of procalcitonin levels in differentiating Gram-negative from Gram-positive sepsis in children aged one month and above admitted in Philippine Children's Medical Center (PCMC) from January 2022 to December 2024.

Methodology: This is a retrospective diagnostic accuracy study on pediatric patients aged one month and above admitted for sepsis in PCMC from January 2022 to December 2024. Inclusion required a positive blood culture for bacterial growth obtained on the same calendar day as procalcitonin level. Patients with non-infectious conditions that could influence procalcitonin level and those with polymicrobial or fungal growth on blood culture were excluded. Descriptive statistics were used to summarize the demographics and laboratory results and the diagnostic accuracy of procalcitonin in differentiating Gram-negative from Gram-positive sepsis were evaluated using the receiver operating characteristic (ROC) curve analysis.

Results: A total of 300 children with sepsis were included, of whom 73% had Gram-negative sepsis and 27% had Gram-positive sepsis. Median procalcitonin levels were not significantly different between Gram-negative and Gram-positive sepsis (p=0.452), although values were higher in Gram-negative cases (7.4 ng/mL [IQR 1.0–29.1] vs 2.2 ng/mL [IQR 0.3–33.0]). ROC analysis identified an optimal cutoff of 0.86 ng/mL, with a sensitivity of 77.3% (95% CI: 71.2% to 82.6%), specificity of 42.5% (95% CI: 31.5%-54.1%) and area under the curve (AUC) of 0.6 indicating poor discriminatory ability. Specific pathogens with the highest observed procalcitonin levels were *E. coli* (29.6 ng/mL, IQR 10.7-83.8), *S. pneumoniae* (21.6 ng/mL, IQR 2.1-87.2), and *Klebsiella* spp. (13.3 ng/mL, IQR 3.4-40.2). Subgroup analysis excluding prior antibiotic exposure showed similar findings, with poor overall discriminatory ability.

Conclusion: Procalcitonin alone cannot reliably differentiate between Gram-negative and Gram-positive sepsis in children. Unlike findings in adult studies, procalcitonin levels in Gram-negative sepsis were not significantly different from those in Gram-positive sepsis. These findings may suggest possible age-related differences in host response, pathogen distribution, and disease severity.

KEYWORDS: procalcitonin, sepsis, children, Gram-negative sepsis, Gram-positive sepsis

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INTRODUCTION

Sepsis is a commonly encountered clinical condition and a frequent cause of admission in pediatric patients. Globally, sepsis in the pediatric population is estimated to have an incidence of 1.2 million cases per year[1] and is responsible for 10-25% of Pediatric Intensive Care Unit (PICU) admissions[2]. It is also a frequent cause of morbidity and mortality, with rates as high as 9-20%,[1] a figure that increases with delayed initiation and/or inappropriate selection of antimicrobial therapy.[3] Especially in the rising threat of antibiotic resistance and

well-documented adverse effects of unnecessary antibiotic exposure, indiscriminate antibiotic use is highly discouraged. Thus, there is a need for a rapid and reliable diagnostic test that not only confirms or excludes sepsis but also provides insight into the likely causative pathogen, thereby supporting timely and appropriate administration of antibiotics. Although blood culture remains to be the gold standard for diagnosis of bloodstream infections, results require at least 24-48 hours and may be falsely negative in 30% of cases.[3,4] Molecular diagnostic techniques such as Polymerase Chain Reaction (PCR) are highly sensitive and specific however are very expensive and require specialized equipment that is not widely available.[3]

Among the serum biomarkers, procalcitonin is a 116-amino acid peptide precursor of the hormone calcitonin normally produced by thyroid C-cells at very low levels in healthy individuals (<0.1 ng/mL).[4,5] During systemic bacterial infections, its production is induced in extra-thyroidal tissues such as the liver, pancreas, kidneys, lungs, intestines, and leukocytes through stimulation by proinflammatory cytokines including interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and interleukin-1β (IL-1β). Unlike the thyroid gland, these sites are unable to convert procalcitonin to calcitonin thereby causing a rapid increase and systemic accumulation of procalcitonin. This occurs within 3-4 hours of bacterial infection, peaks in 6-12 hours, and has a half-life of approximately 24 hours.[3,6] It therefore aids not only in supporting the consideration of bacterial infection but also in monitoring for disease progression and guiding the duration of antibiotic treatment.

A growing number of studies have shown that procalcitonin may be used in distinguishing the likely etiologic pathogen in bloodstream infections. Higher procalcitonin levels have been observed in Gram-negative sepsis compared to Gram-positive sepsis attributed to differential immune activation. The lipopolysaccharide from Gram-negative bacteria strongly activates Toll-like receptor 4 (TLR4) resulting in greater cytokine production and subsequent procalcitonin release. On the other hand, Gram-positive bacteria primarily activate the Toll-like receptor (TLR2) resulting in comparatively weaker cytokine response and less procalcitonin production.[3,5,7–13] However, these studies are mostly done in the adult population and limited data exist in local pediatric populations. Given the physiological differences between children and adults, pediatric patients may exhibit different procalcitonin thresholds. Accordingly, further research is warranted to evaluate whether procalcitonin can reliably differentiate Gram-negative from Gram-positive sepsis in children which would be highly valuable in guiding appropriate antimicrobial therapy.

This study aimed to determine the diagnostic accuracy of procalcitonin in differentiating Gram-negative from Gram-positive sepsis in children aged one month and above admitted in Philippine Children's Medical Center (PCMC) from January 2022 to December 2024. Specifically, it sought to describe the demographic and clinical profile of pediatric patients with culture-confirmed sepsis, assess the association between procalcitonin levels and the Gram stain classification of bacterial pathogens, and evaluate the diagnostic performance using sensitivity, specificity, positive and negative predictive values, likelihood ratios, and the area under the receiver operating characteristic (ROC) curve.

MATERIALS AND METHODS

Study Design and Setting

This is a retrospective diagnostic accuracy study using cross-sectional data conducted on pediatric patients with culture confirmed sepsis admitted in Philippine Children's Medical Center from January 2022 to December 2024.

This study adhered to the ethical principles outlined in the Declaration of Helsinki, Data Privacy Act (2012) and National Ethical Guidelines for Research Involving Human Participants (NEGRIHP 2022). Ethical approval was obtained from Institutional Review-Ethics Committee of the Philippine Children's Medical Center.

Population and Sample Size

The required sample size was calculated using a sample size calculator estimating a single area under the curve (AUC) (https://dishu.page/calculator/) developed by Di Shu and Guanyong Zou (2023). Expecting a 3:7 ratio of negative (Gram-positive sepsis) to positive (Gram-negative sepsis) cases, an anticipated AUC of 0.7, a lower bound confidence limit at 0.6, a level of significance of 5% and assurance probability of 80%, the minimum required sample size was a total of 295 patients. The 3:7 ratio was based on the observed distribution of culture-positive bloodstream infection in the study institution and supported by pediatric data from Li et al. showing predominance of Gram-negative organisms (~65%) over Gram-positive organisms (~35%) in bacteremic patients aged 1 month to 18 years old.[14] Due to the absence of comparable pediatric studies, the anticipated AUC was based on adult studies (0.63-0.95).[3,7,9,10,12,13,15–19] Given this wide range of

reported values, a conservative anticipated AUC of 0.7 was selected.

Eligibility Criteria

Eligible participants included pediatric patients aged one month and above admitted in PCMC from January 2022 to December 2024 with a diagnosis of sepsis. In accordance with the International Pediatric Sepsis Consensus, sepsis was identified by the presence of systemic inflammatory response syndrome (SIRS) alongside a suspected or proven infection. For the purposes of this study, infection was defined as confirmed bacteremia or at least one positive blood culture identifying a bacterial pathogen considered causative of sepsis. Blood culture isolates commonly associated with contamination, including coagulase negative staphylococcus, *Corynebacterium* spp., and other skin flora were included if isolated from at least two separate blood culture sites or if deemed clinically significant based on clinical correlation and physician documentation. Likely contaminants were excluded. SIRS required at least two of the following criteria (one of which must be an abnormal temperature or white blood cell count): (a) core temperature (rectal, bladder, oral or central catheter probe) of > 38.5°C or < 36°C, (b) tachycardia (mean heart rate of >2 standard deviation [SD] above normal for age) or, for children younger than one year, bradycardia (mean heart rate of <10th percentile for age), (c) tachypnea (mean respiratory rate >2 SD for age) or need for mechanical ventilation unrelated to an underlying neuromuscular disease or general anesthesia, (d) peripheral white blood cell (WBC) count elevated or depressed for age or >10% immature neutrophils.[4,20]

Eligibility also required that all samples for blood culture and procalcitonin levels were collected within the same 24-hour calendar day. This window was established to ensure that the procalcitonin level reflected the host's immune response to the specific bloodstream infection identified by the concurrent blood culture while accounting for the known kinetics of procalcitonin during bacterial infection. Patients with non-infectious conditions that could influence procalcitonin levels were excluded. This included a medical history of malignant tumor (small cell lung cancer, bronchial cancer, and medullary thyroid cancer), autoimmune diseases (Still's disease, rheumatoid arthritis, ankylosing spondylitis, systemic lupus erythematosus, vasculitis, or multiple sclerosis), recent major surgery, severe trauma or burns, prolonged cardiogenic shock, and chronic kidney disease. Patients with polymicrobial or fungal growth in blood cultures were also excluded from this study.

Laboratory Examinations

Serum procalcitonin levels were measured using the automatic analyzer B.R.A.H.M.S. PCT sensitive KRYPTOR (Thermo Fisher Scientific) following the manufacturer's instructions. The assay has a functional sensitivity of approximately 0.06 ng/mL and a measuring range of 0.02-50 ng/mL, extendable up to 5000 ng/mL with automatic dilution. Quality control procedures, including regular calibration and use of on-board quality control kits were done following manufacturer recommendations. Insufficient and hemolyzed samples were rejected and repeat extraction was done.

Blood samples that were aseptically collected for culture were inoculated into BacT/Alert culture bottles and incubated in the BacT/Alert 3D Automated Microbial Detection System (bioMérieux). Positive blood cultures were subjected to Gram staining and subcultured onto Blood, Chocolate and MacConkey agar plates. Bacterial isolates were identified using the VITEK 2 Compact system (bioMérieux). These procedures for procalcitonin determination and processing of blood cultures remained consistent throughout the study period.

Data Collection

Charts of patients one month and above admitted for sepsis between January 2022 to December 2024 were reviewed. Data including age, gender, blood culture and procalcitonin result were gathered from patients who met the eligibility criteria. Antibiotic administration prior to sample collection was also noted. To ensure data quality, patient records with missing or incomplete laboratory results or essential identifiers were excluded during the screening process. For children who developed multiple episodes of sepsis during the same hospitalization, only data from the first episode were included provided that the eligibility criteria were met. Patients were classified as Gram-positive or Gram-negative sepsis according to the Gram stain of the causative pathogen identified in blood culture.

Statistical Analysis

Descriptive statistics were used to summarize the demographics and laboratory results. This includes frequency, percentage, mean ± standard deviation and median (interquartile range, IQR). Normal distribution consistency of continuous variables was assessed using Shapiro-Wilk's test. Student's t-test was used for

comparisons of normally distributed variables while Mann–Whitney U test was used for non-normally distributed variables. The chi-square test was used to analyze associations between categorical variables. The diagnostic accuracy was evaluated using receiver operating characteristic (ROC) curve and was described by the following parameters: sensitivity, specificity, positive and negative predictive value (PPV and NPV), positive and negative likelihood ratio (+LR and -LR) and area under the curve (AUC). Youden's index (Youden's index = sensitivity + specificity – 1) was calculated to find the optimal cut-off value for serum procalcitonin level in the differentiation of Gram-positive and Gram-negative sepsis. Results were considered statistically significant when the p-value was ≤ 0.05.

A subgroup analysis was conducted among patients without prior antibiotic exposure to explore its potential influence on procalcitonin levels. The same statistical protocols were done: median procalcitonin levels were compared using the Mann-Whitney U test, and the diagnostic accuracy for differentiating Gram-negative from Gram-positive sepsis was reassessed using ROC curve analysis to determine if discriminatory power improved in the absence of antibiotic exposure.

RESULTS

A total of 300 patients met the inclusion and exclusion criteria (Table 1). Of these, 58% were male and 42% were female with no significant difference in gender distribution between Gram-negative and Gram-positive sepsis groups (p value = 0.697). On the other hand, the mean age was slightly higher in Gram-negative sepsis compared to Gram-positive sepsis (5.8 ± 5.8 years vs 4.4 ± 5.1 years, p =0.044). However, the small mean difference of 1.4 and the wide standard deviations suggest highly heterogeneous age range in both groups hence limited clinical relevance.

Procalcitonin level had a median of 4.9 ng/mL with an interquartile range of 0.7 to 31.4 ng/mL suggesting a wide range of procalcitonin levels within the cohort. Median procalcitonin did not differ significantly between Gram-negative and Gram-positive sepsis (7.4 ng/mL [IQR=1.0-29.1] vs. 2.2 ng/mL [IQR=0.3-33.0]; p=0.452). The median difference was 5.2 ng/mL, indicating a trend toward higher levels in Gram-negative sepsis however the substantial overlap in interquartile ranges suggests limited clinical utility in differentiating Gram-negative from Gram-positive sepsis.

Table 1. Demographic and clinical profile of patients with sepsis admitted in PCMC from January 2022 to December 2024 (N = 300)

Variable	Total (N=300)	Gram-positive sepsis (n=80)	Gram-negative sepsis (n=220)	p value
Gender, n (%)				0.697
Male	174 (58.0)	44 (55.0)	130 (59.1)	
Female	126 (42.0)	36 (45.0)	90 (40.9)	
Age, years	5.4±5.6	4.4 ± 5.1	5.8 ± 5.8	0.044
Procalcitonin, ng/mL	4.9 (0.7-31.4)	2.2 (0.3-33.0)	7.4 (1.0-29.1)	0.452

Note: SD – standard deviation; IQR – interquartile range; data are presented as n (%), mean ± SD, or median (IQR), as appropriate.

Table 2. Procalcitonin levels corresponding to pathogens isolated from children with sepsis

Pathogen	Number of Patients	Procalcitonin, median (IQR)
Gram-negative bacteria		
Klebsiella spp.	54	13.3 (3.4-40.2)
Acinetobacter spp.	32	1.4 (0.3-14.4)
Pseudomonas spp.	29	4.7 (1.3-25.7)
Escherichia coli	26	29.6 (10.7-83.8)
Gram-positive bacteria		
Staphylococcus aureus	36	6.45 (0.3-39.8)
Coagulase negative Staphylococci	11	0.13 (0.03-4.7)
Streptococcus pneumoniae	10	21.6 (2.1-87.2)

Blood cultures had growths of Gram-positive organisms in 26.7% and Gram-negative organisms in 73.3% of patients (Table 1). The most frequent Gram-negative pathogens were *Klebsiella* spp. (n=54), *Acinetobacter* spp. (n=32), *Pseudomonas* spp. (n=29) and *Escherichia coli* (n=26). On the other hand, the most frequent Gram-positive pathogens were *Staphylococcus aureus* (n=36), Coagulase negative Staphylococci (n=11) and *Streptococcus pneumoniae* (n=10). Table 2 shows the varying median values for each pathogen. The organisms with the highest observed median procalcitonin levels were *E. coli* (29.6ng/mL, IQR 10.7-83.8), *S. pneumoniae* (21.6ng/mL, IQR 2.1-87.2) and *Klebsiella* spp. (13.3ng/mL, IQR 3.4-40.2).

To evaluate the diagnostic accuracy of procalcitonin in distinguishing Gram-negative from the Gram-positive sepsis, a receiver operating characteristic (ROC) curve was performed and has identified a procalcitonin cut-off of 0.86 ng/mL (Table 3). At procalcitonin levels below 0.86 ng/mL, results show that 34 of 84 cases (40.5%) had Gram-positive sepsis and 50 (59.5%) had Gram-negative sepsis. In contrast, at procalcitonin levels at or above 0.86 ng/mL, 46 of 216 cases (21.3%) had Gram-positive sepsis and 170 (78.7%) had Gram-negative sepsis, suggesting a trend toward higher procalcitonin level in Gram-negative sepsis. The sensitivity of this cut-off was found to be moderate at 77.3% (95% CI: 71.2% to 82.6%), however the specificity was lower at 42.5% (95% CI: 31.5%-54.1%). The positive predictive value was recorded at 78.7% (95% CI: 72.6%-84.0%), while the negative predictive value stood at 40.5% (95% CI: 29.9%-51.7%). The likelihood ratios (+LR and -LR) were modest at 1.3 and 0.5, respectively.

Table 3. Diagnostic accuracy of procalcitonin level in differentiating Gram-negative from Gram-positive sepsis in children

Procalcitonin	Sensitivity	Specificity	PPV	NPV	+LR	-LR	AUC (95% CI)	P Value
0.86*	77.3	42.5	78.7	40.5	1.3	0.5	0.6 (0.5-0.7)	0.845

* Cutoff identified by ROC analysis; PPV – positive predictive value; NPV – negative predictive value; LR – likelihood ratio; AUC – area under the curve; CI – confidence interval

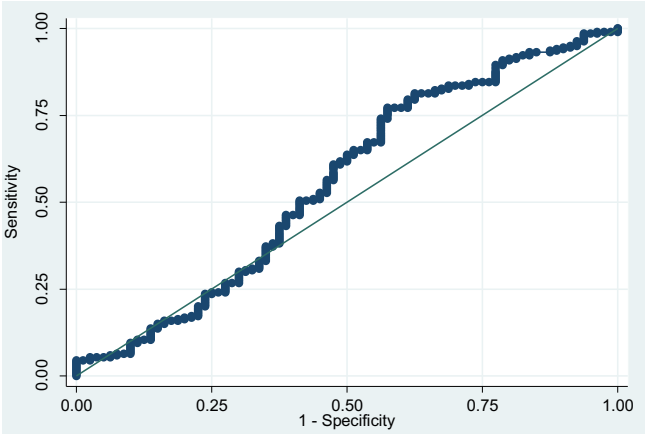

Figure 1. ROC curve of procalcitonin in differentiating between Gram-negative from Gram-positive sepsis

Figure 1 shows the ROC curve of procalcitonin in differentiating Gram-negative from Gram-positive sepsis. The curve lies only slightly above the diagonal line and has an AUC of 0.6 (95% CI: 0.5-0.7, p=0.845).

A subgroup analysis stratified by prior antibiotic exposure was performed to explore its potential influence on procalcitonin levels. Procalcitonin levels were significantly higher in children without prior antibiotic exposure compared to those who had received antibiotics (8.5 ng/mL [IQR 0.7-42.4] vs 2.3 ng/mL [IQR 0.7-17.2]; p=0.013). However, diagnostic performance remained poor. Using a cutoff of 0.5 ng/mL, the sensitivity was 66.2% (95% CI: 53.7-77.2%), specificity was 16.2% (95% CI: 10.3%-23.6%), PPV of 29.2% (95% CI: 22.2-37.1), NPV of 47.7 (95% CI: 32.5-63.3), +LR of 0.78, -LR of 2.08, and the overall AUC was only 0.51 (95% CI: 0.3-0.9, p=0.985) indicating no discriminatory ability. The wide confidence interval for AUC suggests significant imprecision in the estimate, likely owing to the smaller sample size within this specific subgroup.

DISCUSSION

In this single-center pediatric study, results showed that serum procalcitonin levels were higher in Gram-negative sepsis than Gram-positive sepsis, however, the overlap was substantial making the difference not statistically significant. This is contrary to findings seen in similar studies done in adults showing significantly higher procalcitonin levels in Gram-negative sepsis as opposed to Gram-positive sepsis.[3,7,9,10,12,15,17–19,21] Findings in this study, on the other hand, are consistent with pediatric reports. A study done by Nellis et al. (2016) showed that in patients less than 21 years old admitted at a pediatric intensive care unit (PICU) with positive bacterial growth on blood culture (n=27), the median procalcitonin values of Gram-negative sepsis were similar compared to Gram-positive sepsis (15.58 ng/mL, IQR: 0.67-35.01 versus 13.72, IQR: 0.63-34.13).[22] Similarly, a recent study done by Li et al (2025) revealed that in children 1 month to 18 years-old with bloodstream infection (n=98), mean procalcitonin levels in Gram-negative was comparable to Gram-positive sepsis (17.6 ng/mL $\pm$ 40.2 ng/mL versus 11.6 ng/mL $\pm$ 25.3 ng/mL).[14] However, for both studies, direct statistical comparison between the two groups was not performed. While both pediatric studies had small sample sizes, opposing findings compared to adults may suggest that the kinetics of procalcitonin in the pediatric population may differ from those observed in adults.

ROC analysis in this study further highlights the limitations of procalcitonin in differentiating Gram-negative from Gram-positive sepsis in this pediatric population. This showed that using the optimal cutoff of 0.86 ng/mL, the sensitivity was found to be moderate at 77.3% (95% CI: 71.2% to 82.6%), suggesting that ~77% or around 3 out of 4 Gram-negative sepsis cases will be correctly identified. However, the specificity was lower at 42.5% (95% CI: 31.5%-54.1%), suggesting that only ~43% of Gram-positive sepsis will be correctly identified and more than half of Gram-positive sepsis will be falsely identified as Gram-negative.

The predictive values are clinically relevant in decision-making as these reflect the probability that a test result correctly identifies the patient's condition. A PPV of 78.7% in this study suggests that with a procalcitonin level more than the cutoff of 0.86 ng/mL, the chance of having Gram-negative sepsis is approximately 79%. On the other hand, if the procalcitonin level is below the cutoff, there is only a 40.5% chance that the patient does not have Gram-negative sepsis. However, predictive values are highly dependent on disease prevalence. Therefore, the predominance of Gram-negative sepsis in this cohort (73.3%) increases the observed PPV (78.7%) and lowers the NPV (40.5%). To illustrate this effect, recalculating the PPV and NPV using a lower assumed Gram-negative prevalence of 50%, with the same sensitivity and specificity, yields a lower PPV of 57.3% and higher NPV of 65.2%. Hence, the predictive values observed in this study are not generalizable especially in clinical settings where Gram-negative sepsis is less prevalent.

Likelihood ratios describe how much a diagnostic test result changes the probability that a patient has a specific condition and are independent of disease prevalence, making them more stable across different clinical settings. However, since both +LR (1.3) and -LR (0.5) are close to 1, this suggests that procalcitonin has minimal impact in increasing or decreasing the probability of Gram-negative sepsis.

Additionally, the ROC curve in differentiating Gram-negative from Gram-positive sepsis supports the poor discriminatory performance of procalcitonin. Theoretically, as the ROC curve progresses from the bottom left to the top right, it indicates an increase in sensitivity as the threshold for classifying Gram-positive sepsis is lowered. The diagonal line from (0,0) to (1,1) represents a random classifier with no discriminatory ability. The closer the ROC curve is to the top left corner of the graph, the better the model's performance. In this study, the curve lies only slightly above the diagonal line and has an AUC of 0.6 with a 95% confidence interval of 0.5 to 0.7. As this interval includes 0.5, this indicates that procalcitonin is not significantly better than chance in differentiating Gram-negative from Gram-positive sepsis. Overall, although procalcitonin tends to be more elevated in Gram-negative sepsis, its discriminative power in differentiating this from Gram-positive sepsis is poor.

Interestingly, although procalcitonin levels were found to be significantly higher in patients without prior antibiotic exposure (8.5 ng/mL [IQR 0.7-42.4] vs 2.3 ng/mL [IQR 0.7-17.2]; p=0.013), exclusion of patients with prior antibiotic exposure did not improve discriminatory performance of procalcitonin with the AUC further decreasing to 0.51 (95% CI: 0.3-0.9, p=0.985). This may be due to persistent substantial overlap of procalcitonin values between Gram-negative and Gram-positive sepsis. In addition, as the confidence interval for the AUC widened further indicating significant imprecision, the smaller sample size may have limited the ability to detect differences in the diagnostic performance.

Comparing to other similar studies done in adults, although these showed significant differences in procalcitonin levels of Gram-negative versus Gram-positive sepsis, their ROC analyses showed varying results. A study by Yan et al (2017) showed significant difference in median procalcitonin level in Gram-negative sepsis (2.42 ng/mL, IQR: 0.38–15.52) compared to Gram-positive sepsis (0.49 ng/mL, IQR: 0.13–5.89) (P = 0.001), but their ROC analysis showed similar results to this study. With a cut off of 0.495 ng/mL, the sensitivity was only 72.4%, specificity of only 51% and an AUC of only 0.62.[15] Studies with more acceptable AUC, which quantifies the overall ability of the test to discriminate between Gram-negative vs Gram-positive sepsis, ranging from 0.75-0.95, had a wide range of cutoff values from as low as 0.767 ng/mL (Niu, et al.)[12] to as high as 12.88 ng/mL (Sharma, et al).[17] The studies with the highest sensitivity and specificity were those done by Bilgili et al[3] and Wang et al[9] showing cut offs of 1.3 ng/mL (sensitivity: 70.83%, specificity: 84.21%, AUC 0.8) and 4.15 ng/mL (sensitivity: 82%, specificity: 96%, AUC 0.95), respectively. Unfortunately, no comparable ROC analysis has been done in children.

Several factors may explain the discrepancy between pediatric and adult findings. First there are possible age-related differences and comorbid conditions. Neonates were found to have a physiological increase in procalcitonin after birth necessitating the use of different cut-off values for diagnosing bacterial infection in this age group. Beyond the neonatal period and under normal conditions, procalcitonin should not be detectable in the blood hence adult reference ranges are used and applied to children. However, procalcitonin levels in response to bacterial infections may differ between adults and children because of age-related differences in epidemiology, microbiology and immune response in sepsis.[23] Comorbid conditions that affects production and clearance of procalcitonin may also differ in different age groups. Thus, clinicians should interpret a given rise in procalcitonin differently in an infant, child, young adult or geriatric patient.[23] Immune system maturity or response to a particular infection may play a role and may differ between adults and children. A study done by Mustapha et al (2024) on neonates with sepsis also showed no significant difference between Gram-negative versus Gram-positive sepsis with median procalcitonin values of 3.04 ng/mL (IQR 0.96-11.19) and 1.93 ng/mL (0.58-5.11), respectively (p= 0.3),[24] paralleling our findings in older children. In addition, Li et al (2025) also showed mean procalcitonin levels in fungal sepsis in a pediatric cohort to be higher (37.7 $\pm$ 45.9 ng/mL) compared to Gram-negative and Gram-positive sepsis;[14] a finding that contradicts adult studies.

Second, the pathogen distribution may differ in populations and age groups. Adult studies show significantly higher procalcitonin levels in Gram-negative sepsis owing to its activation on the toll-like receptor (TLR) 4 causing release of pro-inflammatory cytokines specifically tumor necrosis factor-alpha (TNF-*a*) and interleukins (IL) 1 and 6 which promotes

production and release of procalcitonin.[5] Although this may still be seen in children, this study showed that Gram-positive organisms may also induce a significant rise in procalcitonin. One such organism is *S. pneumoniae* which produces a cytolytic toxin called pneumolysin which has been suspected to strongly activate TLR4 as well.[5,16] Looking at the procalcitonin levels per organism in this study, the organisms with the highest observed procalcitonin levels were *E. coli* (29.6 ng/mL, IQR: 10.7-83.8), *S. pneumoniae* (21.6 ng/mL, IQR: 2.1-87.2) and *Klebsiella* spp. (13.3 ng/mL, IQR: 3.4-40.2). *S. pneumoniae,* a Gram-positive bacterium, with comparable median procalcitonin values to those of Gram-negative sepsis, comprised 12% of Gram-positive sepsis included in this study. It is also one of the most common bacterial infections in children but seldomly seen in adults. Thomas-Rüddel et al. (2018) likewise found that *Streptococcus* spp., along with *E. coli* and other Enterobacteriaceae, were independently associated with higher procalcitonin concentrations with median values similar to this study: 18.2 ng/mL, 26.8 ng/mL and 24.9 ng/mL respectively.[16] Apart from this, no other adult study showed a significant proportion of *S. pneumoniae* as a causative pathogen. The predominance of pathogens such as *S. pneumoniae*, common in children but less frequent in adult cohorts, may therefore narrow the observed gap between Gram-negative and Gram-positive infections in the pediatric age group.

Lastly, disease severity and extent of inflammation may influence procalcitonin levels. Lautz et al. (2016) demonstrated that higher illness severity in children with sepsis correlated with elevated procalcitonin, regardless of the pathogen.[25] This suggests that elevated procalcitonin may also reflect host response and illness severity rather than just the specific pathogen type. This should be taken into consideration especially in the setting of a referral children's hospital where cases tend to be more severe, complex and in which multiple factors such as organ dysfunction or other sources of inflammation may affect procalcitonin levels and may contribute to the lack of Gram-negative/Gram-positive distinction in this study.

While there is a growing number of adult studies on the use of biomarkers in pathogen differentiation, to our knowledge there are no similar pediatric studies published locally or globally. This study highlights the importance of generating further studies in the pediatric population as findings from adult populations may not be applicable to children.

The main limitation of this study is its retrospective design. The analysis was dependent on the accuracy, completeness and quality of the data or information available on the chart. In addition, although the study tried to control potential confounders that could affect procalcitonin levels, including performing a subgroup analysis to address prior antibiotic exposure, unrecognized factors present at the time of admission may still have influenced procalcitonin levels. Another limitation is the absence of data on the interval between symptom or infection onset and sample collection, which is known to greatly affect the procalcitonin level as levels are known to rise within 3-4 hours, peak in 6-12 hours and has a half-life of 24 hours. Similarly, the 24-hour window between procalcitonin and blood culture sample collections may have included patients in different phases of procalcitonin response including both rising and falling levels. Although this was intended to ensure temporal association between the identified bloodstream infection and measured procalcitonin response, this may introduce variability in the procalcitonin values independent of the pathogen type and consequently reduce the discriminatory precision of procalcitonin in differentiating Gram-negative from Gram-positive sepsis in this study. Furthermore, this study was conducted in a single tertiary pediatric referral hospital, which may limit the generalizability of the findings. Pathogen distribution and disease severity in this setting may not reflect those seen in primary or secondary care facilities.

CONCLUSION

The results of this study provide locally relevant preliminary data on the use of procalcitonin in differentiating Gram-negative from Gram-positive sepsis in a local pediatric population. While a procalcitonin cutoff of 0.86 ng/mL showed moderate sensitivity in differentiating Gram-negative from Gram-positive sepsis, its low specificity and AUC of 0.60 means that it is poorly reliable for this purpose. The study also showed that, unlike in adult studies, procalcitonin levels in Gram-negative sepsis had no significant difference from that in Gram-positive sepsis. This is a valuable observation for future studies in children and highlights potential age-related differences in host response, pathogen distribution and disease severity which may affect procalcitonin levels. These results emphasize the need for caution in interpreting procalcitonin levels for guiding empiric antimicrobial treatment in pediatric sepsis.

RECOMMENDATIONS

Given the limitations of this study, further prospective multicenter cohort studies involving a larger and more diverse pediatric population, with clearly defined inclusion and exclusion criteria, with the exclusion of patients with prior antibiotic exposure, and possibly integrating illness severity scoring is recommended to better understand the patterns of procalcitonin levels in pediatric sepsis. A standardized timing of sample collection (possibly within a 6–12-hour window) to better account for procalcitonin kinetics and reduce temporal variability may also be considered. In addition, given that procalcitonin alone showed poor discriminatory power, future studies exploring procalcitonin combined with other biomarkers, such as CRP, IL-6, hematologic indices, or integration into clinical algorithms may improve diagnostic accuracy for early pathogen identification in pediatric sepsis.

Conflicts of Interest

None declared.

REFERENCES

1. Massaud-Ribeiro L, Silami PHNC, Lima-Setta F, Prata-Barbosa A. Pediatric Sepsis Research: Where Are We and Where Are We Going? *Front. Pediatr* [Internet]. 2022 Feb;10:829119. Available from: https://pubmed.ncbi.nlm.nih.gov/35223703/ DOI: 10.3389/fped.2022.829119
2. Mau LB, Bain V. Antimicrobial Therapy in Pediatric Sepsis: What Is the Best Strategy? *Front Pediatr* [Internet]. 2022 Feb 15;10:830276. Available from: www.frontiersin.org DOI: 10.3389/fped.2022.830276
3. Bilgili B, Haliloğlu M, Aslan MS, Sayan İ, Kasapoğlu US, Cinel İ. Diagnostic accuracy of procalcitonin for differentiating bacteraemic gram-negative sepsis from gram-positive sepsis. *Turk J Anaesthesiol Reanim* [Internet]. 2018 Feb;46(1):38-43. Available from: https://pubmed.ncbi.nlm.nih.gov/30140499/ DOI: 10.5152/TJAR.2017.88965.
4. Cherry J, Kaplan S, Harrison G, Steinbach W, Hotez P, Williams J. Feigin and Cherry's Textbook of Pediatric Infectious Diseases. 9th Edition. Philadelphia: Elsevier Inc; 2025. p.2776-2786
5. Xu HG, Tian M, Pan SY. Clinical utility of procalcitonin and its association with pathogenic microorganisms. *Crit Rev Clin Lab Sci* [Internet]. 2022 Mar;59(2):93–111. Available from https://pubmed.ncbi.nlm.nih.gov/34663176/ DOI: 10.1080/10408363.2021.1988047
6. Samsudin I, Vasikaran SD. Clinical Utility and Measurement of Procalcitonin. *Clin Biochem Rev*. 2017 Apr; 38(2):59-68. Available from: https://pubmed.ncbi.nlm.nih.gov/29332972/
7. Leli C, Ferranti M, Moretti A, Al Dhahab ZS, Cenci E, Mencacci A. Procalcitonin levels in gram-positive, gram-negative, and fungal bloodstream infections. *Dis Markers* [Internet]. 2015; 2015: 701480. Available from: https://pubmed.ncbi.nlm.nih.gov/25852221/ DOI: 10.1155/2015/701480
8. Tsao YT, Tsai YH, Liao WT, Shen CJ, Shen CF, Cheng CM. Differential Markers of Bacterial and Viral Infections in Children for Point-of-Care Testing. *Trends Mol Med* [Internet]. 2020 Dec;26(12):1118–32. Available from: https://pubmed.ncbi.nlm.nih.gov/33008730/ DOI: 10.1016/j.molmed.2020.09.004.
9. Wang D, Tao X, Guo W, Liu H, Cheng S, Wang J. The diagnostic ability of procalcitonin to differentiate Gram-negative bacteria from Gram-positive bacteria and fungal bloodstream infections in critically ill patients. *Eur J Inflamm* [Internet]. 2019;17:1-6. Available from: https://journals.sagepub.com/doi/10.1177/2058739219841469 DOI: 10.1177/2058739219841469
10. Li S, Rong H, Guo Q, Chen Y, Zhang G, Yang J. Serum procalcitonin levels distinguish Gram-negative bacterial sepsis from Gram-positive bacterial and fungal sepsis. *Res Med Sci* [Internet]. 2016 Jun 14;21:39. Available from: https://pubmed.ncbi.nlm.nih.gov/27904585/ DOI: 10.4103/1735-1995.183996.
11. Dambroso-Altafini D, Menegucci TC, Costa BB, Moreira RRB, Nishiyama SAB, Mazucheli J, et al. Routine laboratory biomarkers used to predict Gram-positive or Gram-negative bacteria involved in bloodstream infections. *Sci Rep* [Internet]. 2022 Sep 14;12(1): 15466 Available from: https://pubmed.ncbi.nlm.nih.gov/36104449/ DOI: 10.1038/s41598-022-19643-1.
12. Niu D, Huang Q, Yang F, Tian W, Li C, Ding L, et al. Serum biomarkers to differentiate Gram-negative, Gram-positive and fungal infection in febrile patients. *J Med Microbiol* [Internet]. 2021 Jul;70(7). Available from: https://pubmed.ncbi.nlm.nih.gov/34259621/ DOI: 10.1099/jmm.0.001360.
13. Guo Y, Zhou Y, Feng Hu Q, Yao J, Wang H. Procalcitonin Is a Marker of Gram-Negative Bacteremia in Patients With Sepsis. *Am J Med Sci* [Internet]. 2015 Jun;349(6):499–504. Available from: https://pubmed.ncbi.nlm.nih.gov/25992537/ DOI: 10.1097/MAJ.0000000000000477.
14. Li Y, Li M, Lin C, Tang W, Tang Q, Cheng F. Exploring the clinical value of procalcitonin, c-reactive protein, white blood cell count, and neutrophil-to-lymphocyte ratio in the early diagnosis of bloodstream infections in children. *BMC Pediatr* [Internet]. 2025 Jan 24;25(1):62. Available from: https://pubmed.ncbi.nlm.nih.gov/39856637/ DOI: 10.1186/s12887-025-05402-4.
15. Yan ST, Sun LC, Jia HB, Gao W, Yang JP, Zhang GQ. Procalcitonin levels in bloodstream infections caused by different sources and species of bacteria. *Am J Emerg Med* [Internet]. 2017 Apr;35(4):579–583. Available from: https://pubmed.ncbi.nlm.nih.gov/27979420/ DOI: 10.1016/j.ajem.2016.12.017.
16. Thomas-Rüddel DO, Poidinger B, Kott M, Weiss M, Reinhart K, Bloos F. Influence of pathogen and focus of infection on procalcitonin values in sepsis patients with bacteremia or candidemia. *Crit Care* [Internet]. 2018 May;22(1):128. Available from: https://pubmed.ncbi.nlm.nih.gov/29753321/ DOI: 10.1186/s13054-018-2050-9.
17. Sharma V, Patro S, Choudhary A, Pradhan S, Pathi B. Combined Predictive Value of Serum Procalcitonin and Platelet Indices in Differentiating Gram-Negative and Gram-Positive Bloodstream Infections. *Cureus* [Internet] 2025 Sep;17(9):e91390. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC12486313/ DOI: 10.7759/cureus.91390.
18. Koizumi Y, Sakanashi D, Ohno T, Nakamura A, Yamada A, Shibata Y, et al. Plasma procalcitonin levels remain low at the onset of gram-positive bacteremia regardless of severity or the presence of shock: A retrospective analysis of patients with

detailed clinical characteristics. *J Microbiol Immunol Infect* [Internet]. 2021 Dec;54(6):1028–1037. Available from: https://pubmed.ncbi.nlm.nih.gov/32893142/ DOI: 10.1016/j.jmii.2020.08.015.

19. Gao Q, Li Z, Mo X, Wu Y, Zhou H, Peng J. Combined procalcitonin and hemogram parameters contribute to early differential diagnosis of Gram-negative/Gram-positive bloodstream infections. *J Clin Lab Anal.* 2021 Sep;35(9):e23927. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC8418508/ DOI: 10.1002/jcla.23927.
20. Goldstein B, Giroir B, Randolph A; International Consensus Conference on Pediatric Sepsis. International pediatric sepsis consensus conference: definitions for sepsis and organ dysfunction in pediatrics. *Pediatr Crit Care Med* [Internet]. 2005 Jan;6(1):2-8. Available from: https://pubmed.ncbi.nlm.nih.gov/15636651/ DOI: 10.1097/01.PCC.0000149131.72248.E6. PMID: 15636651.
21. Leng Y, Chen C, Zhang Y, Luo C, Liu B. Ability of serum procalcitonin to distinguish focus of infection and pathogen types in patients with bloodstream infection. *Ann Transl Med* [Internet]. 2019;7(7):135–143. Available from: https://atm.amegroups.org/article/view/24917/23458 DOI: 10.21037/atm.2019.03.24
22. Nellis ME, Pon S, Giambrone AE, Coleman NE, Reiss J, Mauer E, et al. The Diagnostic Accuracy of Serum Procalcitonin for Bacteremia in Critically Ill Children. *Infect Dis Clin Pract (Baltim Md)* [Internet]. 2016 Nov;24(6):343–347. Available from: https://pubmed.ncbi.nlm.nih.gov/27857510/ DOI: 10.1097/IPC.0000000000000432.
23. Downes KJ, Fitzgerald JC, Weiss SL. Utility of Procalcitonin as a Biomarker for Sepsis in Children. *J Clin Microbiol* [Internet]. 2020 Jun 24;58(7):e01851-19. Available from: https://pubmed.ncbi.nlm.nih.gov/32350044/ DOI: 10.1128/JCM.01851-19.
24. Mustapha SS, Adedeji IA, Dogara AB, Yusuf MS, Aliyu S, Abdulkadir I. The role of Procalcitonin in neonatal sepsis-examining facts and misconceptions. *Journal of Clinical Neonatology* [Internet]. 2024 Apr;13(2):35–41. Available from: https://journals.lww.com/jocn/fulltext/2024/13020/the_role_of_procalcitonin_in_neonatal.1.aspx DOI:10.4103/jcn.jcn_7_24
25. Lautz AJ, Dziorny AC, Denson AR, O'Connor KA, Chilutti MR, Ross RK, et al. Value of Procalcitonin Measurement for Early Evidence of Severe Bacterial Infections in the Pediatric Intensive Care Unit. *J Pediatr* [Internet]. 2016 Dec;179:74-81.e2. Available from: https://pubmed.ncbi.nlm.nih.gov/27587074/ DOI: 10.1016/j.jpeds.2016.07.045.