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Early Detection of Blood Culture Positivity in Pediatric Cardiac Surgery Patients Using Immature Granulocyte Percentage and Absolute Count

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ABSTRACT

Introduction: This study assesses immature granulocyte count as an early predictor of blood culture positivity in pediatric cardiac patients. Early diagnosis of sepsis is crucial but challenging. Blood culture is the gold standard; however, obtaining results takes 48-72 h. The study compares these indicators with other predictive markers of sepsis. **Methods:** This retrospective study analyzed data from 200 pediatric patients to assess the use of immature granulocyte count as an early predictor of blood culture positivity in pediatric cardiac surgery patients with sepsis. The patients were divided into two groups based on blood culture results: positive and negative. A complete blood count was conducted for both groups, including immature granulocyte count and demographic information. The data were collected for two periods: 24-48 h before the blood culture and 24 hours after. The study aimed to evaluate the diagnostic utility of immature granulocyte count and compare it with other established predictive markers of sepsis. The blood counts were performed using SYSMAX XN1000. **Results:** The study observed higher immature granulocyte counts in patients with culture-positive results during period 2 diagnosis (P<0.001). No significant differences were found in other lab parameters between the two groups. The receiver operating characteristic (ROC) curve analysis showed that an immature granulocyte count ≥ 90 was helpful in predicting blood culture positivity in pediatric cardiac surgery patients with sepsis. **Conclusion:** Our study reveals that the Absolute Immature Granulocyte Count and Immature Granulocyte percentage (IG%) significantly increase within 24-48 hours of positive blood cultures compared to negative cases.

INTRODUCTION

Congenital heart disease (CHD) is one of the most prevalent birth defects, affecting approximately 1% of all children worldwide. Surgical intervention is recommended for repairing heart defects in affected children, as it can significantly enhance their long-term well-being.

Sepsis after pediatric cardiac surgery leads to significant morbidity and mortality, placing a substantial financial burden on healthcare systems [1].

Timely diagnosis of sepsis plays a crucial role in achieving a high treatment success rate. Nevertheless, the definitive diagnosis of sepsis poses challenges, impeding timely treatment. While blood culture is widely regarded as the gold standard for sepsis diagnosis, it often necessitates 48-72 h to yield results [2].

In pediatric patients, parameters such as manual band cell count, left shift, and the immature-to-total neutrophil (I/T) ratio are frequently employed to predict bacterial infections.

The 'left shift' in a Sysmex hematology analyzer is identified by the immature granulocyte count (IG#), which encompasses the neutrophil fraction comprising promyelocytes, myelocytes, and metamyelocytes [3]. This fraction is hypothesized to reflect the initial bone marrow response to infection, and its quantification holds potential as a predictive marker for sepsis. The automated IG# test is easily accessible, cost-effective, and highly reproducible, rendering it a straightforward and reliable diagnostic tool.

This study aims to evaluate the diagnostic utility of immature granulocyte count (IG#) and percentage (IG%)

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as early predictors of blood culture positivity after pediatric cardiac surgery and to compare their performance with other established markers for predicting sepsis. Establishing IG as a biomarker for the prompt and precise diagnosis of sepsis in the Intensive Care Unit (ICU) setting can enhance sepsis management and minimize antibiotic utilization.

MATERIAL AND METHODS

This study received approval from the Institutional Ethics Committee of the U.N. Mehta Institute of Cardiology and Research Centre (UNMICRC/Patho/2023/01), a tertiary care super-specialty hospital in the western state of Gujarat, India.

Inclusion criteria. This retrospective study included data from 200 pediatric patients under 18 years old who underwent cardiac surgery to treat congenital heart disease. The patients were admitted to the institute between October 2022 and December 2022.

Exclusion criteria. The study excluded patients with hematological malignancies, immunodeficiency diseases, and individuals receiving immunosuppressive medications. Patients who developed sepsis and experienced mortality within 24 h of its onset were excluded.

The sample size 200 was determined using Raosoft software, considering a 5% significance level. The study achieved a statistical power of 80% and utilized a 95% confidence interval. The study employed a non-probability consecutive sampling technique. Blood cultures were collected from the pediatric post-cardiac surgical unit when infection was clinically suspected after surgery. These cultures were processed following the clinical and laboratory standards recommended by the institute.

The patients were classified into two groups: those with positive blood culture results and those without. Group I comprised patients with positive blood culture results, whereas Group II comprised patients with negative blood culture results. Period 1 referred to the 24- to 48-h period preceding blood culture collection, whereas period 2 referred to the 24- to 48-h period after blood culture collection. During periods 1 and 2, comprehensive blood count data were collected from both groups, encompassing total leukocyte count (TLC), absolute neutrophil count (ANC), neutrophil-to-lymphocyte ratio (NLR), percentage of immature granulocytes (IG%), absolute count of immature granulocytes (IG#), and platelet count. Additionally, demographic information, including age, gender, hospital stay, and duration of invasive line usage was recorded. Blood counts were conducted utilizing the SYSMEX XN-1000 instrument.

Statistical analysis. Data analysis was conducted using IBM SPSS version 21 and MedCalc software. Normally distributed data were presented as mean ± standard deviation (SD), while non-normally distributed data were presented as the median. Categorical variables were expressed as frequencies and percentages. Mean differences were assessed using Student's t-test, while median differences were evaluated using the Mann-Whitney U test. Receiver operating characteristic (ROC) curves were constructed for each marker in each period, and the area under the curve (AUC) was calculated to evaluate the tests' validity and accuracy. Optimal cutoff values for each biomarker were determined using Youden's index, followed by calculating the corresponding sensitivity and specificity measures. The diagnostic odds ratio (DOR) was calculated to identify the most accurate predictive biomarker for each study period, with a significance level of 0.05 used to determine statistical significance.

RESULTS

Data were collected from 200 patients, and the study included 195 patients who underwent pediatric cardiac surgery. Five patients were excluded from the analysis due to unavailable data. Among the population, 100 patients tested positive for culture (group I), while 95 tested negative (group II). The age of the study population ranged from 100 to 300 months. There were no significant differences in age between the two groups (P=0.227). The male-to-female ratios were comparable, with 58 male and 42 female patients in group I and 60 male and 35 female patients in group II (P=0.552). No significant differences were observed in other laboratory parameters, including hemoglobin, total count, and absolute neutrophil counts. The neutrophil-lymphocyte ratio (NLR) and platelet-lymphocyte ratio (PLR) are presented in Table 1.

At the time of diagnosis in period two, patients in culture-positive Group I exhibited significantly higher values of absolute IG and IG% (P<0.001 for both) compared to other groups (Table 2).

In the univariate logistic regression analysis considering continuous parameters, both absolute IG and IG% they demonstrated higher odds ratios for sepsis prediction compared to other parameters (odds ratio: 1.90, P < 0.001 and odds ratio: 1.00, P = 0.013, respectively) (Table 3).

In the subsequent ROC analysis, IG# and IG% exhibited higher AUC values than other parameters, with AUCs of 0.654 and 0.648, respectively.

Figure 1 depicts the ROC curve analysis for Absolute IG in Period 2, yielding an AUC of 0.654 (P = 0.001), a sensitivity of 50%, and a specificity of 77.9%. The optimal cut-off point for Absolute IG in Period 2 was 0.80.

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Table 1. Comparison of different parameters between the groups

	Group 1 culture +	Group 2 culture -	*P*-value
Age in months (median)	4	5	0.227
HB period 1 (mean ± SD)	12.50 ± 2.42	12.79 ± 2.51	0.427
HB period 2 (mean ± SD)	11.85 ± 2.02	11.86 ± 2.28	0.972
Total count period 1 (mean ± SD)	13730.7 ± 6456.4	13415.3 ± 5028.0	0.704
Total count period 2 (mean ± SD)	14222.2 ± 7304.2	13400.7 ± 6183.9	0.398
Absolute neutrophil count period 1 (mean ± SD)	8518.17 ± 5809.2	7943.4 ± 4643.2	0.447
Absolute neutrophil count period 2 (mean ± SD)	9555.07 ± 6578.3	8785.09 ± 5339.4	0.373
Neutrophil-lymphocyte ratio period 1 (median)	2.81	2.71	0.227
Neutrophil-lymphocyte ratio period 2 (median)	2.86	3.15	0.716
Platelet lymphocyte ratio period 1 (median)	1.97	2.03	0.526
Platelet lymphocyte ratio period 2 (median)	1.53	1.45	0.888

Table 2. Comparison of absolute IG and IG% between groups

	Group 1 culture +	Group 2 culture -	*P*-value
Ig% period 1 (median)	0.50	0.50	0.977
Ig% period 2 (median)	0.85	0.50	<0.001
Ig count period 1 (median)	60	50	0.976
Ig count period 2 (median)	105	50	<0.001

Table 3. Logistic regression analysis to identify the optimal predictive marker

			Regression analysis			
			Score	difference	Significance	Odds Ratio
Step 0	Variables	IG period 1	.708	1	.400	0.44
		IG period 2	12.204	1	.000	1.905
		IG period 1	.025	1	.874	1.000
		IG period 2	6.124	1	.013	1.000

Figure 2 illustrates the ROC curve analysis for IG count in Period 2, exhibiting an AUC of 0.648 (P = 0.002), a sensitivity of 55%, and a specificity of 71.6%. The determined cut-off point for absolute IG in Period 2 is presented in Table 4.

Blood culture-positive patients exhibited a higher frequency of Immature Granulocyte Percentage (IG%) values exceeding the 0.8 cut-off threshold compared to blood culture-negative patients within 24-48 h following blood culture collection. The ANC, length of Central Traumatic Red Cell Resuscitation (CTRR) stay, and duration of urinary catheterization were significantly more significant in the blood culture-positive group compared to the blood culture-negative group of patients. Among the blood culture-positive group of patients, granulocyte counts exceeding 90 demonstrated substantial elevations in total counts, ANC, CTRR stay, and duration of urinary catheterization compared to the blood culture-negative group (Table 5).

DISCUSSION

Neutrophils play a critical role in immune response by releasing essential regulatory cytokines, chemokines, and leukotrienes within white blood cells [4]. The presence of immature granulocytes in the peripheral blood, a left shift in granulocyte distribution, signifies an active bone marrow response to bacterial infection. These cells are classified based on their morphology, including promyelocytes, myelocytes, metamyelocytes, and band forms, through microscopic examination of blood films [5]. However, the acquisition of these measurements has posed significant challenges. Fortunately, recent technological advancements have made automated hematology analyzers affordable and user-friendly, enabling accurate identification and counting of immature granulocytes. White blood cells (WBCs) were incorporated as a fundamental component in the definition of sepsis by Bone *et al*. (1992) [6]. The Sepsis-3 criteria, the latest framework for defining sepsis [7], do not incorporate WBCs or neutrophils as part of their diagnostic criteria. Nevertheless, neutrophils, particularly the occurrence of a left shift, retain a pivotal role in the pathobiology of sepsis and are recognized as among the earliest indicators of infection and the body's response to sepsis. Several studies, including those by Buoro *et al*. (2015), Bourne *et al*. (2013), Mac Queen *et al*. (2016), and others, have substantiated the utilization of immature granulocyte count through the Sysmex XN 1000 system as an indicator of left shift and, consequently, sepsis [8, 9, 10].

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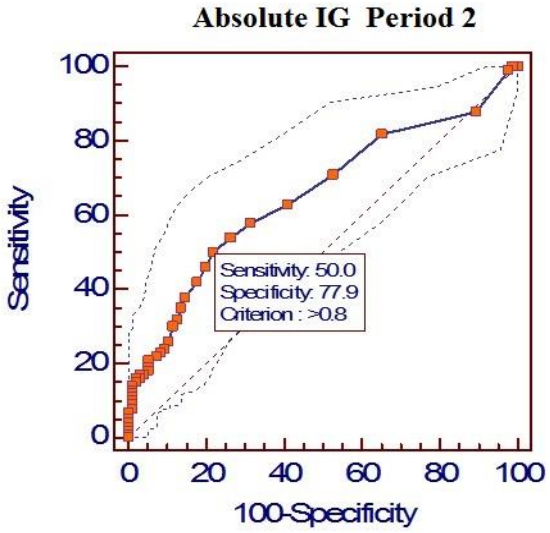


Fig. 1. ROC curve analysis for absolute IG in Period 2, represented by AUC value

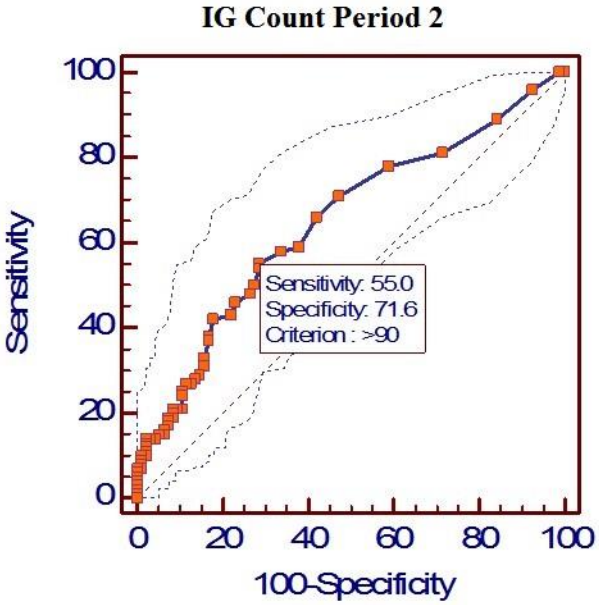


Fig. 2. ROC curve analysis for IG count in Period 2, represented by the AUC value

In pediatric patients, the absolute count and percentage of immature granulocytes are valuable indicators of infection or inflammation severity. Identifying accurate predictors for positive blood cultures is imperative in managing infections among cardiac patients, given the seriousness and inherent challenges associated with these infections.

While blood culture remains the gold standard for sepsis diagnosis, its results are frequently delayed by 48-72 h. Blood cultures can identify bacteria, fungi, or yeasts at concentrations as low as one colony-forming unit (CFU) per 10 ml of blood. Nevertheless, conventional cultures necessitate up to 96 h of incubation time and exhibit limited sensitivity towards slow-growing, intracellular, and fastidious microorganisms, particularly in patients undergoing antimicrobial treatment [11].

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Table 4. The ROC curve analysis of IG and IG% for sepsis prediction

Variables	AUC	95% CI	Cut-off value	Sensitivity	Specificity	*P*-value
IG % period 2	0.654	0.58 – 0.72	0.8	50	77.9	0.001
IG Count period 2	0.648	0.57 – 0.71	90	55	71.6	0.002

Table 5. Comparison of various parameters with their respective cut-off values

IG%	>0.8	<0.8	*P*-value
Period 2 total count	16295.1 ± 8564.6	11788.6 ± 4446.5	0.0017
Period 2 ANC count	11627.6 ± 7674.6	7067.9 ± 3719.8	0.0004
CTRR stay	13.82 ± 2.06	6.0 ± 4.8	<0.001
SICU stay	5.8 ± 6.4	4.3 ± 3.7	0.208
Total LOS	30.58 ± 17.06	26.11 ± 21.15	0.274
Et tube days	10.19 ± 10.09	5.38 ± 17.8	0.117
Urinary catheter days	12 ± 7.4	5.6 ± 3.34	<0.001
Absolute IG	**>90**	**<90**	***P*-value**
Period -1 total count	14892.9 ± 6921.3	12125.7 ± 5432.0	0.033
Period -2 total count	16943.1 ± 7956.0	10464.7 ± 3986.3	<0.001
Period -2 Anc count	11955.5 ± 7248.8	6159.2 ± 3312.1	<0.001
CTRR stay	12.84 ± 13.60	6.45 ± 5.50	0.006
SICU stay	5.76 ± 6.45	4.3 ± 3.30	0.1970
Total LOS	30.32 ± 16.99	26 ± 21.63	0.294
Et tube days	10.88 ± 7.	6.4 ± 5.18	<0.001
Urinary catheter days	11.46 ± 7.65	7.17 ± 8.08	0.011

Despite an estimated positive predictive value of cultures surpassing 95%, the overall positivity rate in sepsis can be as low as 30-40%, even with meticulous adherence to standard procedures, appropriate collection of blood volume, and substantial clinical suspicion of sepsis [11, 12, 13].

Our study aimed to explore potential associations between blood culture positivity and immature granulocyte counts obtained within the initial 24 h of blood sample collection. This approach holds the potential for early prediction of blood culture outcomes before the availability of a definitive report. Ultimately, this approach reduces the turnaround time from blood culture collection to reporting by enabling early prediction of positive blood culture results based on immature granulocyte counts. Consequently, it can potentially enhance treatment outcomes regarding morbidity and mortality.

Several studies have examined the association between immature granulocyte counts (IG# and IG%) and blood culture positivity in pediatric patients with cardiac disease [14, 15]. In a study published in the Journal of Clinical Laboratory Analysis in 2018, it was observed that patients with positive blood cultures exhibited significantly higher immature granulocyte counts (IG# and IG%) compared to those with negative blood cultures. Furthermore, the study revealed that a combined evaluation of immature granulocyte counts (IG# and IG%) provides a more accurate prediction of blood culture positivity than either parameter alone.

Consistent with a study published in Interact Cardiovasc Thorac Surg in 2019 [15], elevated immature granulocyte counts (IG# and IG%) were observed in blood cultures, yielding positive results. The study identified a cut-off value of 0.3 for immature granulocyte percentage (IG%) with a sensitivity of 81.5% and specificity of 76.7%. Our study identified a cut-off value of 0.8 for immature granulocyte percentage (IG%), demonstrating a sensitivity of 50% and specificity of 77.9%. Similarly, a cut-off value 90 for immature granulocyte count (IG#) was determined, with a sensitivity of 55% and specificity of 71.6%.

It is essential to acknowledge that immature granulocyte counts (IG# and IG%) can provide diagnostic assistance in identifying infection; however, they lack specificity and may be elevated in other conditions. Therefore, integrating clinical and laboratory parameters alongside immature granulocyte counts is crucial for accurately diagnosing infection.

Immature granulocyte counts, encompassing both the absolute count (IG#) and percentage (IG%), hold significant potential as indicators of infection in pediatric patients with cardiac disease. Moreover, combining these parameters may enhance the accuracy of predicting positive blood cultures.

Our study showed that immature granulocyte counts, including the absolute count (IG#) and percentage (IG%), were markedly higher within the first 24 h among blood culture-positive cases than blood culture-negative cases.

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