



EVALUATION OF IMMATURE GRANULOCYTES AS AN EARLY PREDICTOR OF NEONATAL SEPSIS AS COMPARED TO BLOOD CULTURE

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ABSTRACT Neonatal sepsis is the commonest and important cause for morbidity and mortality of neonates in developing countries. Various studies have documented high level of immature granulocytes count in early stages of sepsis. The aim of this study was to assess sensitivity & specificity of immature granulocyte count % by automated hematology analyser (sysmex XN 550) in detecting culture proven neonatal sepsis at Assam Medical College Hospital. A total of 90 neonates were selected with clinical signs & symptoms associated with neonatal sepsis & with risk factors for sepsis. 4 ml of peripheral venous blood was collected under aseptic conditions. 1 ml blood was sent for estimation of serum CRP. 2 ml blood was sent for estimation of TLC, ANC, IG% by automated hematology analyser. 1 ml blood was sent for culture and sensitivity testing. Out of 90 cases studied, 49 cases (54.44%) yielded positive blood culture & 41 cases (45.56%) were culture negative. The sensitivity and specificity of serum CRP in detecting neonatal sepsis were 73.47% and 65.85% respectively. 32 cases with culture positivity had IG count >0.5% whereas 17 cases with culture positivity had IG count ≤ 0.5%. For an IG cut off 0.5% the sensitivity was 65.31% and specificity 75.61%. Immature granulocytes count % had significant association (P value 0.00027) with onset of neonatal sepsis. It is a reliable indicator of culture proven neonatal sepsis & consumes less reporting time

KEYWORDS :

INTRODUCTION:

Neonatal sepsis is the commonest and the most important cause for the morbidity and mortality of neonates in developing countries like India. The signs and symptoms of sepsis in neonates are subtle and non-specific which makes it difficult to diagnose clinically. Timely diagnosis of sepsis in neonates is critical as the illness can progress more rapidly when compared to adults. The first 28 days of life – the neonatal period – represent the most vulnerable time for a child's survival. In 2016, 2.6 million deaths, or roughly 46% of all under-five deaths, occur during this period. This translates to 7000 newborn deaths every day. The majority of the neonatal deaths are concentrated in the first day and week, with about 1 million dying on the first day and close to one million dying within the next six days.⁽¹⁾ Measurement of cytokines, acute phase proteins, cell surface antigens and bacterial genomes are used for the early diagnosis of sepsis either alone or in combination. The granulocytic "shift to left" reflects marrow response to bacterial infection, and this increases the amount of circulating immature granulocytes in peripheral circulation.

AIM: Evaluation of immature granulocytes as an early predictor of neonatal sepsis as compared to blood culture.

OBJECTIVE: To assess the importance of immature granulocytes in the early diagnosis of culture proven neonatal sepsis. To compare the automated hematology analyser immature granulocytes count % with the findings of blood culture report.

MATERIAL AND METHOD:

A total of 90 neonates (<28 days of age) were selected who presented to NICU with clinical signs and symptoms associated with neonatal sepsis⁽²⁾ and had presence of risk factors⁽³⁾ for sepsis. Neonates with major congenital anomalies and those who received antibiotics were excluded from study. 4 ml of peripheral venous blood was collected under aseptic conditions. 1 ml blood was sent for estimation of serum c-reactive protein (CRP). 2 ml blood was sent for estimation of TLC, ANC and IG% by automated hematology analyser. 1 ml blood was sent for culture and sensitivity testing. Statistical analysis was carried out to SPSS for Windows version 21.0 and Microsoft excel 2010. For all analysis the statistical significance was fixed at 5% level of significance (p value < 0.05). Diagnostic statistics viz. Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Value and Accuracy have been computed to find the correlation of blood investigations with culture report. IG% were estimated through

fluorescence flowcytometry method by the analyser, as the immature granulocytes contain higher nucleic acid content compared to the mature counterpart. Automated immature granulocyte cut off reference value was taken as 0.5% in cases for neonatal sepsis, with values greater pointing towards sepsis⁽⁴⁾.

RESULTS:

Out of 90 cases studied, 49 cases (54.44%) yielded a positive blood culture and 41 cases (45.56%) were culture negative. Higher proportion of culture positive cases were in age group 0-3 days (75.52%) compared to cases in the age group 4-28 days (24.48%). 31 males (63.26%) were culture positive while 18 females (36.74%) were culture positive out of total 49 culture positive cases. In the 49 culture positive cases, 37 cases (75.52%) had low birth weight while 12 cases (24.48%) had normal birth weight. The most common clinical features are respiratory distress (55.10%) followed by seizures (22.44%) and refusal to feed (20.4%). The most common risk factors were low birth weight (75.51%), birth asphyxia (69.38%) and prematurity (28.57%). The most common organisms isolated in blood culture were Klebsiella Pneumonia 20.40% followed by Acinetobacter Baumannii 20.40% and Enterococcus Faecalis 10.21%. 32 cases with culture positivity had IG count >0.5% whereas 17 cases with culture positivity had IG count ≤ 0.5%. For an IG cut off value of 0.5% the sensitivity was 65.31% and specificity 75.61%. The positive predictive value and negative predictive value were 76.19% and 64.58% respectively. The P-value was found to be statistically significant (0.00027) in predicting culture proven sepsis.

CORRELATION OF IG COUNT % WITH CULTURE REPORT

IG COUNT (%)	CULTURE POSITIVE	CULTURE NEGATIVE	TOTAL	p value
IG > 0.5%	32(TP)	10 (FP)	42	0.0027
IG ≤ 0.5%	17(FN)	31(TN)	48	
TOTAL	49	41	90	
STATISTIC	VALUE	95% CI		
Sensitivity	65.31%	50.36% to 78.33%		
Specificity	75.61%	59.70% to 87.64%		
Disease Prevalence	54.44%	43.60% to 64.98%		

Positive Predictive Value (PPV)	76.19%	64.26% to 85.06%
Negative Predictive Value (NPV)	64.58%	54.47% to 73.54%

DISCUSSION:

Immature granulocytes are white blood cells that have not fully developed before being released from the bone marrow into the blood. These cells are normally only present in the bone marrow because they are precursors of neutrophils, the predominant type of white cells in blood. The automated hematology analyzer separates neutrophil maturation into 2 groups. Mature granulocytes consist of segmented neutrophils and bands, and are also reported as the absolute neutrophil count. Immature granulocytes include metamyelocytes, myelocytes, and promyelocytes. Historically, Bands have been considered less mature and were correlated with a shift toward immature cells (left shift); however, Bands are fully functional phagocytes and based on their ability to fight infection, are classified as mature neutrophils.(5)(6)

We also compared the findings of IG% with other sepsis screening parameters:

PARAMETERS	SENSITIVITY (%)	SPECIFICITY (%)	PPV (%)	NPV (%)
IG Value >0.5%	65.31	75.61	76.19	64.58
Serum CRP >1mg/dl	73.47	65.85	72.00	67.50
Reduced ANC	34.69	85.36	73.91	52.24
Leucopenia <5000/ μ l	20.43	87.88	66.67	48.00

The specificity of IG% was found higher than serum CRP, while serum CRP had higher sensitivity. Leucopenia and Neutropenia had the highest specificity among all the test.

COMPARISON BETWEEN SOME PREVIOUS STUDIES WITH PRESENT STUDY:

Study	Nigro et al 2005 ⁽⁷⁾	Cimenti et al 2012 ⁽⁸⁾	Pavare et al 2018 ⁽⁹⁾	Zeng et al 2020 ⁽¹⁰⁾	Present study
IG % cut off value	0.5%	1.3%	0.45%	0.5%	0.5%
Sample size	181 infants (0 to 253 days)	133 neonates	258 (1month to 18years)	256 (0 days to 12 year)	54 neonates
sensitivity	33%	67%	66%	59%	65.31%
specificity	88%	88%	84%	79%	75.61%
PPV	64%	67%	90%	53%	76.19%
NPV	68%	27%	51%	80%	64.58%

The present study had a sensitivity and specificity of 65.31% and 75.61% respectively which is almost similar to findings of Pavare et al(5) and Cimenti et al(4).The specificity was high compared to the sensitivity,which was common finding in almost all the studies.The present study found IG% to have a high positive predictive value (76.19%) and low negative predictive value (64.58%) similar to findings of Pavare et al(5).The variation in sensitivity and specificity in different studies is subject to variation in blood sampling time, the extent of infection and inflammation and exposure to antibiotics.

In severe bacterial infections, the large amount of bacteria and their products in blood stimulate the production of enormous quantities of several cytokines, notably TNF α and IL-1. These causes proliferation of precursor cells in bone marrow caused by increased production of colony stimulating factors. Also there is accelerated release of cells from post mitotic reserve pool leading to rise in the number of immature granulocytes in blood.(11) Preterm babies and low birth weight babies are susceptible to infection due to deficient immune system (Decreased IgG, immature epithelial barriers etc).

CONCLUSION:

Immature granulocytes count % was found to have a significant association with the onset of neonatal sepsis. It is a reliable indicator of

culture proven neonatal sepsis and consumes very less reporting time.The use of molecular markers in diagnosis of neonatal sepsis will further reduce the neonatal mortality rate. The sensitivity and specificity of IG% as a diagnostic test in neonatal sepsis is almost comparable with serum CRP. Serum CRP is a known sepsis screening parameter.

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