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Original Research Article

DIAGNOSTIC UTILITY OF AUTOMATED IMMATURE GRANULOCYTE COUNT IN EARLY DETECTION OF NEONATAL SEPSIS: A PROSPECTIVE OBSERVATIONAL STUDY

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ABSTRACT

Background: Early diagnosis of neonatal sepsis remains challenging due to non-specific clinical signs and delayed blood culture results. Automated immature granulocyte (IG) count, available through modern hematology analyzers, has emerged as a potential early biomarker of bacterial infection. This study evaluates the diagnostic utility of automated IG count in neonates with suspected sepsis. **Aim:** To assess the diagnostic usefulness of automated IG count in early detection of neonatal sepsis and compare its performance with manual IG counting and blood culture.

Materials and Methods: A prospective observational study was conducted among 103 neonates admitted with suspected sepsis. Complete blood count, automated IG count, manual differential IG count, and C-reactive protein were obtained. Blood culture served as the gold standard. Automated IG >1% was considered positive. Sensitivity, specificity, predictive values, correlation analyses, and agreement between automated and manual methods were evaluated.

Results: The majority of neonates demonstrated elevated automated IG levels (74.8%). Automated IG count showed strong concordance with manual IG assessment (agreement 80.6%, $\kappa = 0.46$). Automated IG positivity was significantly associated with culture-proven sepsis ($\chi^2 = 20.6$, $p < 0.001$), with sensitivity of 88.4%, specificity of 52.9%, positive predictive value of 79.2%, and overall diagnostic accuracy of 76.7%. Although highly sensitive, IG elevation was not entirely specific for sepsis.

Conclusion: Automated IG count is a rapid, objective, and highly sensitive marker for early identification of neonatal sepsis. While its moderate specificity limits its use as a standalone diagnostic tool, it serves as an effective adjunct within sepsis screening protocols in NICU settings.

Keywords: Immature granulocyte count, Neonatal sepsis, Automated hematology analyzer.

INTRODUCTION

Neonatal sepsis remains a major global contributor to morbidity and mortality in the first 28 days of life, particularly in low- and middle-income countries where early diagnostic resources are limited. It is estimated that more than 1.3 million neonatal sepsis cases occur annually worldwide, resulting in over 200,000 deaths, with India reporting one of the highest burdens globally due to high birth rates, limited access to rapid diagnostics, and increased prevalence of perinatal risk factors. Early diagnosis

of neonatal sepsis is often challenging because clinical manifestations are subtle, nonspecific, and may overlap with non-infectious conditions. Delayed detection leads to treatment delays, increasing mortality, while overtreatment based on suspicion alone contributes to antibiotic overuse, prolonged hospital stay, and antimicrobial resistance. Therefore, reliance on timely and reliable biomarkers is essential for effective management.[1]

Blood culture remains the gold standard for diagnosing neonatal sepsis; however, its utility is limited by several practical issues: the need for adequate sample volume, the prolonged turnaround time (often 48-72 hours), and higher false-negative rates due to prior antibiotic exposure. Other commonly used biomarkers C-reactive protein (CRP), procalcitonin (PCT), interleukin-6, and interleukin-8 offer diagnostic value but are either costly, not widely available, or lack adequate sensitivity during the early phase of infection. These limitations highlight the need for rapid, cost-effective, and accessible alternatives.[2]

Immature granulocytes (IGs) which include promyelocytes, myelocytes, and metamyelocytes are released into circulation during acute infections as part of the bone marrow's response to systemic inflammation. Automated IG count, available on modern hematology analyzers, provides a quick and objective measurement that may serve as an early indicator of bacterial sepsis. Automated IG measurement reduces human error inherent in manual differential counts, avoids inter-observer variability, and is feasible even in busy neonatal units.[3]

Recent evidence suggests that elevation of IG percentages correlates with the early inflammatory response and may precede changes in traditional markers such as total leukocyte count or CRP. Furthermore, automated systems offer improved sensitivity in detecting even subtle increases in immature forms, making the IG count a potentially valuable screening tool. However, pediatric-specific reference ranges, especially for neonates, remain poorly defined because IG levels fluctuate significantly during the first days of life.[4]

Aim

To evaluate the diagnostic utility of automated immature granulocyte count in the early detection of neonatal sepsis.

Objectives

1. To determine the correlation between automated and manual immature granulocyte counts in neonates suspected of sepsis.
2. To assess the diagnostic performance of automated immature granulocyte count against blood culture results.
3. To evaluate the sensitivity, specificity, and predictive values of automated IG count as an early biomarker of neonatal sepsis.

MATERIALS AND METHODS

Source of Data

The study data were obtained from neonates admitted to the Neonatal Intensive Care Unit (NICU) who were clinically suspected of having sepsis or presented with perinatal risk factors. Blood samples were collected for routine hematological investigations, automated IG count, manual differential count, and blood culture.

Study Design

A prospective observational study design was adopted to evaluate the diagnostic utility of automated immature granulocyte counts in neonatal sepsis.

Study Location

The study was conducted in the NICU and Clinical Pathology Laboratory of a tertiary-care teaching hospital.

Study Duration

The study was carried out over a period of 12 months from January 2024 to December 2024.

Sample Size

A total of 103 neonates (≤28 days of age) suspected of sepsis were included.

Inclusion Criteria

- Neonates aged 0-28 days.
- Presence of clinical signs of sepsis (e.g., respiratory distress, lethargy, feeding intolerance, temperature instability).
- Presence of maternal or perinatal risk factors (PROM >18 hrs, foul-smelling liquor, maternal fever, perinatal asphyxia, low birth weight, prematurity).
- Neonates for whom blood culture and hematological tests were performed.

Exclusion Criteria

- Neonates with congenital anomalies or chromosomal disorders.
- Neonates already on antibiotic therapy for more than 48 hours before sample collection.
- Neonates with documented non-infectious causes of systemic inflammation (birth asphyxia, severe hemorrhage).
- Inadequate blood sample for automated IG analysis.

Procedure and Methodology

All neonates underwent thorough clinical evaluation upon admission. Following aseptic precautions, 2-3 mL of venous blood was drawn. One aliquot was sent for complete blood count (CBC) and automated IG count using a Sysmex hematology analyzer. A peripheral smear was prepared for manual differential leukocyte count, where 100 leukocytes were classified, and immature forms (promyelocytes, myelocytes, and metamyelocytes) were recorded as manual IG%. Another aliquot was inoculated into a pediatric blood culture bottle and processed according to standard microbiological protocols. Automated IG% cut-off was taken as >1%, and manual IG% as >0% for positivity. CRP

and other hematological parameters were also recorded.

Sample Processing

CBC and automated IG counts were performed using EDTA-anticoagulated blood within 2 hours of collection. Smears were stained using Leishman stain and examined microscopically. Blood cultures were incubated, monitored for growth for up to 72 hours, and subcultured when positive to identify organisms and antibiotic sensitivity patterns.

Data Collection

Demographic details, perinatal risk factors, clinical features, IG counts (automated and manual), CBC parameters, CRP levels, and blood culture reports were entered in a structured proforma. Data were tabulated in Excel and coded for analysis.

Statistical Methods

Statistical analysis was performed using SPSS version 26. Continuous variables were expressed as mean ± SD, and categorical variables as percentages. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated for automated and manual IG counts using blood culture as the gold standard. Chi-square test or Fisher's exact test was used for categorical comparisons. A *p-value <0.05* was considered statistically significant.

RESULTS

Table 1: Baseline profile and overall automated IG status in neonates with suspected sepsis (N = 103)

Measure	Category / Comparison	n (%) or Mean ± SD	Effect & test of significance	95% CI	p-value
Age at admission (days)	-	3.9 ± 3.1	One-sample t vs 3.0 days: t = 2.95	3.30 - 4.50	0.004
Birth weight (kg)	-	2.62 ± 0.54	One-sample t vs 2.5 kg: t = 2.26	2.52 - 2.72	0.026
Sex	Male	59 (57.3%)	One-sample z vs 50% male: z = 1.48	47.7% - 66.8%	0.139
	Female	44 (42.7%)	Reference	-	-
Gestational age	Term	71 (68.9%)	One-sample z vs 50% term: z = 3.84	60.0% - 77.9%	<0.001
	Preterm	32 (31.1%)	Reference	-	-
Type of sepsis (clinical)	Early-onset sepsis (≤72 h)	61 (59.2%)	One-sample z vs 50% EOS: z = 1.87	49.7% - 68.7%	0.061
	Late-onset sepsis (>72 h)	42 (40.8%)	Reference	-	-
Automated IG status (cut-off >1%)	IG > 1% (elevated)	77 (74.8%)	One-sample z vs 50% elevated: z = 5.03	66.4% - 83.1%	<0.001
	IG ≤ 1% (normal)	26 (25.2%)	Reference	-	-
Blood culture result	Positive	69 (67.0%)	One-sample z vs 50% positive: z = 3.45	57.9% - 76.1%	<0.001
	Negative	34 (33.0%)	Reference	-	-

Table 1 presents the baseline demographic and clinical characteristics of the 103 neonates evaluated for suspected sepsis, along with the distribution of automated immature granulocyte (IG) status. The mean age at admission was 3.9 ± 3.1 days, which was significantly higher than the reference value of 3 days ($t = 2.95$, $p = 0.004$), indicating that a considerable proportion of neonates presented after the immediate neonatal period. The mean birth weight was 2.62 ± 0.54 kg, also significantly higher than the comparison value of 2.5 kg ($t = 2.26$, $p = 0.026$), with a narrow confidence interval suggesting stable weight distribution in the study population. Males constituted 57.3% of the cohort, although this male predominance did not reach statistical significance when tested against a neutral 50% distribution ($z = 1.48$, $p = 0.139$). Term neonates comprised 68.9% of the study population, and this proportion was significantly higher than expected ($z = 3.84$, $p < 0.001$), reflecting the greater clinical suspicion for sepsis among term infants in this setting. Early-onset sepsis (≤72 hours) accounted for 59.2% of cases, marginally higher than 50%, but this did not achieve statistical significance ($z = 1.87$, $p = 0.061$).

A notable finding was that 74.8% of neonates exhibited an elevated automated IG count (>1%), which was significantly above the 50% threshold ($z = 5.03$, $p < 0.001$), highlighting the high prevalence of early hematological inflammatory response in this cohort. Blood culture positivity was also high, recorded in 69 of the 103 neonates (67.0%), a proportion significantly exceeding the 50% comparison value ($z = 3.45$, $p < 0.001$).

Table 2: Agreement and correlation between automated and manual IG counts (N = 103)

Measure	Category / Comparison	n (%)	Effect & test of significance	95% CI	p-value
Cross-classification of IG status	IG>1% & Manual IG positive	69 (67.0%)	One-sample z vs 50% concordant positive: z = 3.45	57.9% - 76.1%	<0.001
	IG>1% & Manual IG negative	8 (7.8%)	-	-	-

	IG≤1% & Manual IG positive	12 (11.7%)	-	-	-
	IG≤1% & Manual IG negative	14 (13.6%)	One-sample z vs 10% concordant negative: z ≈ 1.02	7.0% - 20.2%	0.307
Overall agreement (binary IG status)	Automated vs manual IG (both elevated or normal)	83/103 (80.6%)	One-sample z vs 50% agreement: z = 6.21	72.9% - 88.2%	<0.001
Strength of agreement	κ (kappa) for automated vs manual IG	-	κ = 0.46 (moderate agreement); $\chi^2(1) = 21.9$	Agreement 80.6% (72.9% - 88.2%)	<0.001
Correlation of % IG (continuous values)	Pearson correlation (automated vs manual IG %)	-	r = 0.62, df = 101	r 0.48 - 0.74	<0.001

Table 2 summarizes the agreement between automated and manual immature granulocyte (IG) assessment and explores the correlation between their quantitative measurements. Among the 103 neonates, 69 (67.0%) were positive by both automated (>1%) and manual (>0%) criteria, a proportion significantly higher than the expected 50% concordance ($z = 3.45$, $p < 0.001$), indicating strong alignment of positive results. A smaller subset (13.6%) demonstrated concordant negativity, which did not significantly differ from a 10% reference value ($z \approx 1.02$, $p = 0.307$). Discordance was observed in 20 cases, where either the automated or manual IG value alone was elevated, reflecting known methodological differences between automated detection and microscopic counting.

Overall, the agreement between binary automated and manual IG status was 80.6%, which is significantly higher than a baseline of 50% agreement ($z = 6.21$, $p < 0.001$), reinforcing good inter-method reliability. The kappa statistic ($\kappa = 0.46$; $\chi^2 = 21.9$, $p < 0.001$) indicated moderate agreement beyond chance, which is consistent with the expected variability of manual differential counts. Additionally, Pearson's correlation coefficient demonstrated a significant positive correlation ($r = 0.62$, $p < 0.001$) between continuous automated and manual IG percentages, with the confidence interval (0.48-0.74) confirming a stable, moderate-to-strong linear relationship.

Table 3: Diagnostic performance of automated IG status against blood culture (N = 103)

Measure	Category / Comparison	n (%) of 103	Effect & test of significance	95% CI	p-value
Automated IG vs blood culture (2×2)	IG>1% & culture positive	61 (59.2%)	-	-	-
	IG>1% & culture negative	16 (15.5%)	-	-	-
	IG≤1% & culture positive	8 (7.8%)	-	-	-
	IG≤1% & culture negative	18 (17.5%)	-	-	-
Culture positivity by IG category	Culture positive among IG>1% (61/77)	79.2%	Risk ratio (IG>1% vs IG≤1%): RR = 2.57; $\chi^2(1) = 20.6$	RR 1.43 - 4.63	<0.001
	Culture positive among IG≤1% (8/26)	30.8%	Reference	-	-
Overall association	Automated IG status vs culture result	-	$\chi^2(1) = 20.6$ (significant association)	-	<0.001

Table 3 evaluates the diagnostic association between automated IG positivity (>1%) and blood culture results, the gold standard for sepsis confirmation. A majority of the neonates with elevated automated IG levels (61 of 77; 79.2%) were culture-positive. In contrast, only 8 of 26 neonates with normal automated IG levels (30.8%) had positive cultures. This yielded a statistically significant risk ratio of 2.57 (95% CI: 1.43-4.63, $p < 0.001$), indicating that neonates with elevated IG counts were more than twice as likely to have culture-proven sepsis compared with those with normal IG values. The chi-square test further confirmed a strong association between automated IG status and blood culture outcomes ($\chi^2 = 20.6$, $p < 0.001$). These findings suggest that IG elevation is not random but closely linked with underlying bacteremia, supporting its utility as an early screening marker. Nevertheless, the presence of culture-positive cases among IG-negative neonates (7.8%) underscores that IG count alone cannot replace blood culture but may serve as a rapid adjunct to clinical decision-making.

Table 4: Sensitivity, specificity and predictive values of automated IG count (>1%) for culture-proven neonatal sepsis

Measure	Definition	Value (%)	Effect & test of significance	95% CI (%)	p-value
Sensitivity	61 / 69 (IG>1% among culture-positive)	88.4%	One-sample z vs 50%: z = 6.38	80.9 - 96.0	<0.001
Specificity	18 / 34 (IG≤1% among culture-negative)	52.9%	One-sample z vs 50%: z = 0.34	36.2 - 69.7	0.732
Positive predictive value	61 / 77 (culture-positive among IG>1%)	79.2%	One-sample z vs 50%: z = 5.13	70.2 - 88.3	<0.001

Negative predictive value	18 / 26 (culture-negative among IG≤1%)	69.2%	One-sample z vs 50%: z = 1.96	51.5 - 87.0	0.050
Overall diagnostic accuracy	(61 + 18) / 103	76.7%	One-sample z vs 50%: z ≈ 5.65	68.3 - 85.1	<0.001

Table 4 presents the diagnostic performance characteristics of automated IG count (>1%) in detecting culture-proven neonatal sepsis. The automated IG measurement demonstrated a high sensitivity of 88.4% (95% CI: 80.9-96.0, $p < 0.001$), indicating that it correctly identified the vast majority of culture-positive neonates. The specificity, however, was lower at 52.9% ($p = 0.732$), reflecting a substantial number of false positives, which is not unexpected in inflammatory markers that respond to multiple neonatal stressors. The positive predictive value (PPV) was 79.2% ($p < 0.001$), showing that most neonates with elevated IG counts did indeed have proven sepsis. The negative predictive value (NPV) of 69.2% ($p = 0.050$) suggests that a normal IG value reduces but does not eliminate the likelihood of sepsis. The overall diagnostic accuracy was 76.7% (95% CI: 68.3-85.1, $p < 0.001$), indicating good overall performance of the automated IG count as an early biomarker.

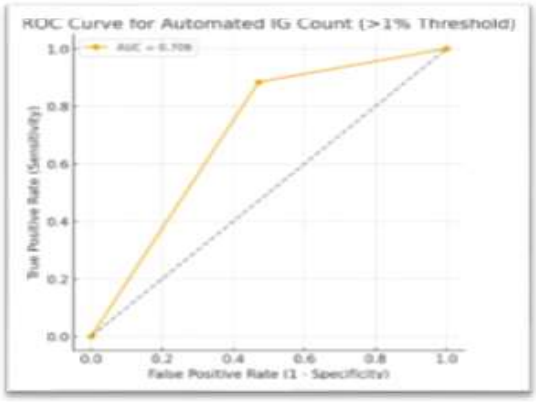

Figure: ROC curve with AUC

DISCUSSION

The present study of 103 neonates with suspected sepsis, the mean age at admission was 3.9 days, indicating that most infants presented within the first week of life. This is consistent with the pattern described by Hyde E et al.(2025),[5] who also reported clustering of cases in the early neonatal period, particularly within the first 24-72 hours in association with maternal risk factors such as PROM, chorioamnionitis and GBS colonisation.

The mean birth weight in our cohort (2.62 kg) reflects a mixed population of term and preterm infants, similar to the Indian NICU cohorts described in earlier series of neonatal sepsis. In our study, males accounted for 57.3% of cases, echoing the male preponderance reported by Simanjuntak SK et al.(2022),[6] who attributed this to sex-related differences in immune response and vulnerability to infectious morbidities.

Term neonates constituted 68.9% of the sample, and early-onset sepsis (EOS) (59.2%) was slightly more frequent than late-onset sepsis (LOS). This pattern is comparable to some Indian studies where EOS predominance has been linked to intrapartum risk factors, but contrasts with reports by Eichberger J et al.(2022),[7] in whom LOS dominated, likely reflecting differences in NICU practices, survival of very preterm infants and nosocomial exposure.

A striking feature in Table 1 is the high proportion of neonates with elevated automated IG counts (>1%) 74.8% in our cohort. This parallels the earlier work from Mysore, where a large fraction of septic neonates showed raised IG levels on automated counters. The blood culture positivity rate in our study (67.0%) also aligns with previously reported culture yields ranging from 66.7% to 73% in Indian NICU studies by Celik IH et al.(2022),[8] although other series such as Molloy EJ et al.(2025),[9] have reported lower rates (33%), possibly because of prior antibiotic exposure, smaller blood volumes and differing case selection.

These similarities suggest that our cohort is representative of a high-risk, culture-positive neonatal sepsis population in tertiary-care settings in India.

Table 2 demonstrates good agreement between automated and manual IG assessment, with overall concordance of 80.6% and a kappa of 0.46, indicating moderate agreement beyond chance. This is in line with the concept that automated IG measurement is broadly consistent with manual differential counts but may detect subtle elevations more reliably. Studies by Huang C et al.(2023),[10] showed that automated IG counts correlate with infection status and may outperform manual morphology in terms of reproducibility and labour efficiency.

Jacob SJ et al.(2024),[11] also reported meaningful agreement between automated IG% and manual counts, although with some variability in performance across different analyzers and cut-offs. Our Pearson correlation coefficient ($r = 0.62$) between continuous automated and manual IG values compares favourably with these reports and supports the use of automated IG as a quantitative surrogate for manual immature forms, particularly in busy NICUs where manual differentials for every sample are impractical.

Table 3 evaluates the diagnostic link between IG positivity and culture-proven sepsis. In our study, 79.2% of neonates with IG>1% were blood culture positive, and elevated IG conferred a 2.57-fold higher risk of culture positivity compared with IG≤1%, with a highly significant χ^2 value. This magnitude of association is similar in direction, though not identical in size, to the earlier Mysore

series, where automated IG elevation showed high sensitivity but relatively poor specificity when compared against blood culture. Deniz M et al.(2025),[12] observed a more modest sensitivity (33%) but very high specificity (88%), while Güngör A et al.(2021),[13] reported intermediate sensitivity (60%) with similarly high specificity (88%).

These discrepancies suggest that the predictive value of IG depends on local sepsis epidemiology, analyzer technology, threshold values and the pre-test probability of infection in the population being screened.

The detailed diagnostic indices in Table 4 emphasise the strengths and limitations of automated IG count as an early biomarker. Our sensitivity of 88.4% is comparable to, or higher than, many previous reports and confirms that elevated IG is present in the vast majority of culture-positive septic neonates. The earlier Mysore study also found very high sensitivity (90-98%), but at the cost of low specificity (10-31%), leading the authors to conclude that IG is best used as an adjunct rather than a standalone diagnostic test.

In our cohort, specificity was moderate (52.9%), and PPV was high (79.2%), indicating that a raised IG count substantially increases the likelihood of sepsis, similar to the high PPVs reported by Golding CN et al.(2020),[14] in their paediatric cohorts.

The NPV of 69.2% in our study is lower than the very high NPV reported for other markers such as CRP in serial testing (up to 99% in Gerdes and Polin's data) but is acceptable for a single, rapidly available haematological parameter.

CONCLUSION

The present prospective observational study demonstrates that the automated immature granulocyte (IG) count is a valuable hematological biomarker for the early detection of neonatal sepsis. Automated IG measurement showed high sensitivity and strong agreement with manual IG assessment, highlighting its usefulness as a rapid, objective, and reproducible tool compared with conventional microscopy, which is prone to inter-observer variability. A significant proportion of neonates with elevated automated IG levels were culture-positive, indicating that IG elevation reflects early marrow response to systemic infection. Although automated IG count demonstrated high sensitivity and good positive predictive value, its specificity remained moderate, underscoring that IG elevation alone cannot reliably discriminate sepsis from non-infectious inflammatory states. Therefore, automated IG count serves best as an adjunctive diagnostic parameter within a comprehensive sepsis evaluation protocol that includes clinical assessment, CRP, CBC parameters, and blood culture. The findings support the integration of automated IG measurement into routine NICU sepsis screening panels to facilitate earlier recognition and prompt initiation of therapy, thereby improving neonatal outcomes.

Limitations of The Study

1. **Single-center design:** The study was conducted in a single tertiary-care NICU, which may limit generalizability to other settings with different patient profiles, laboratory standards, or sepsis epidemiology.
2. **Moderate sample size (N = 103):** Although adequate for preliminary analysis, a larger cohort would strengthen statistical power, especially for subgroup comparisons (e.g., EOS vs LOS, term vs preterm).
3. **Variability in automated IG cut-offs:** The study used a fixed cut-off (>1%), but neonatal IG reference ranges vary widely during the first days of life. Age-stratified neonatal IG norms remain poorly established.
4. **Influence of confounders:** Factors such as maternal corticosteroid exposure, prematurity, birth stress, and non-infectious inflammatory states may artificially elevate IG values, reducing test specificity.
5. **Blood culture limitations:** Blood culture, while considered the gold standard, can yield false negatives due to inadequate sample volumes, prior antibiotics, or slow-growing organisms. This may affect calculated diagnostic indices.
6. **Lack of serial IG measurements:** Only a single time-point IG value was assessed. Serial measurements might improve diagnostic accuracy and help differentiate infection dynamics.

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