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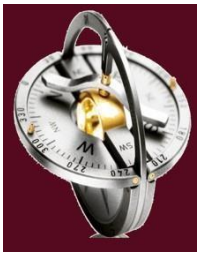

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Original Research

The Role of Neutrophil Surface Markers CD64 and CD11b in the Clinical and Epidemiological Diagnosis and Prognosis of Neonatal Sepsis

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Abstract

Background: Neonatal sepsis remains a major global health burden, contributing substantially to neonatal mortality and long-term neurodevelopmental impairment. Early diagnosis is essential, yet current diagnostic tools–including blood culture and conventional acute-phase reactants–are limited by delayed turnaround times, low sensitivity in early infection, and the challenges of minimal blood volume in neonates. Neutrophil activation markers such as CD64 (nCD64) and CD11b (nCD11b), measurable through rapid flow cytometry, have emerged as promising biomarkers. ***Materials and Methods:*** This study is a comparative clinical analyses of neonatal sepsis performed in high-volume neonatal intensive care units (NICUs). The study focuses on the diagnostic accuracy of flow cytometric assessment of nCD64 and nCD11b as determined by Receiver Operating Characteristic (ROC) curve analysis.1 The population includes neonates, defined as infants aged 0 to 28 days, who were prospectively enrolled and classified into two main categories: the Sepsis Group (Case Group) and the Non-Sepsis Group (Control Group). ***Results:*** Analysis of culture data within the sepsis cohorts highlights a significant epidemiological finding: the majority of clinically defined sepsis cases (56.7%) yield negative blood cultures.3 This high culture-negative rate underscores the vital clinical necessity for rapid and accurate surrogate biomarkers like nCD64. Since CD64 reflects the host immune response to bacterial p roducts, it can confirm the diagnosis of clinical sepsis, allowing for confident initiation of critical care and antibiotic protocols even when the causative organism fails to be isolated via the gold standard.2 Among the positive blood cultures (comprising 43.3% of cases), the most prevalent organisms identified were Gram-negative bacteria, with Klebsiella accounting for 26.7% of positive isolates, followed by E. Coli and Staphylococcus aureus (each at 6.7%), and Staphylococcus Hemolyticus3 (3.3%).3 The dominance of Gram-negative pathogens provides mechanistic support for the effectiveness of CD64, as their lipopolysaccharide (LPS) components are potent activators of the innate immune cascade responsible for CD64 upregulation. ***Conclusion:*** Neutrophil CD64, quantified as the percentage of positive cells (nCD64%), is a highly accurate, sensitive, and rapid diagnostic modality for neonatal sepsis. Its robust performance (AUC 0.894) statistically surpasses that of nCD11b (AUC 0.405) and hs-CRP (AUC 0.586) as a standalone marker

Keywords: *Neonatal sepsis, Neutrophil CD64, Neutrophil CD11b, Flow cytometry, Early diagnosis, Biomarkers*

Introduction

1.1 The Critical Burden and Diagnostic Imperative of Neonatal Sepsis

Neonatal sepsis remains a profound global public health challenge, directly contributing to high rates of neonatal mortality and long-term neurodevelopmental morbidities among survivors.1 Rapid diagnosis and immediate initiation of appropriate antimicrobial therapy are critical steps necessary to mitigate these adverse consequences. However, current diagnostic protocols are often hindered by fundamental limitations. Clinical presentation in neonates is often non-specific, mimicking non-infectious conditions, while the traditional diagnostic gold standard, the blood culture, suffers from a lengthy turnaround time of 24 to 48 hours, often yielding delayed or false-negative results.2 Conventional acute-phase reactants, such as C-reactive protein (CRP), also frequently lack the necessary sensitivity or specificity during the critical early stages of infection.2 Due to the low blood volume of preterm and ill infants, an ideal sepsis biomarker must be feasible using a minimal blood sample (around 50 µl to 100µl) and offer a rapid turnaround time (TAT) of approximately 1 to 2 hours.2

1.2 Neutrophil Activation and the Basis for Surface Biomarker Upregulation

Systemic bacterial infection triggers an immediate and dynamic activation of the innate immune system, where circulating neutrophils play a central role. During this process, neutrophils rapidly upregulate specific cell surface receptors in response to pro-inflammatory signaling molecules. Flow cytometry has emerged as a powerful technological advancement, enabling the quantitative and rapid assessment of these cell surface activation markers in whole blood with minimal processing.2 Among the most studied markers are neutrophil CD64 (nCD64) and neutrophil CD11b (nCD11b), whose expression levels directly correlate with the degree of systemic inflammation and host respo nse to the infectious insult..

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1.3 Mechanistic Role of Neutrophil CD64

CD64, also known as Fc-gamma receptor 1, is the high-affinity receptor for monomeric IgG-type antibodies.4 This receptor is indispensable for mediating efficient phagocytosis and the intracellular killing of microbes that have been opsonized.4 In a healthy, resti ng state, CD64 expression on the surface of neutrophils is typically low or undetectable. However, its expression is rapidly and robustly induced by pro-inflammatory cytokines, notably interferon released during active bacterial infection.5 The superior diagnostic specificity observed for nCD64, compared to other general inflammatory markers, is attributed to this physiological mechanism: the substantial magnitude of upregulation from a minimal baseline expression level, which signifies a targeted, high-alert immunological state against specific bacterial challenges. This kinetic profile allows nCD64 to serve as a powerful discriminator, distinguishing true bacterial sepsis from other non-infectious causes of inflammation or stress that might trigger less specific immune responses.4

1.4 Mechanistic Role of Neutrophil CD11b (ITGAM)

CD11b, also referred to as Integrin alpha M or complement receptor 3 A is primarily involved in regulating leukocyte adhesion to the vascular endothelium and subsequent migration into inflamed tissues.4 This molecule plays a crucial role in mediating inflammation by regulating leukocyte mobility.4 CD11b is known to be upregulated on blood neutrophils in patients experiencing Systemic Inflammatory Response Syndrome (SIRS) or sepsis, representing a phenotype synonymous with cell activation.5 Despite its involvement in the innate immune response, its clinical utility in diagnosing early neonatal sepsis is frequently reported as being inferior to that of nCD64, suggesting differences in the timing, magnitude, or consistency of its expression kinetics in peripheral blood during the early phases of the disease.2

Materials and Methods

2.1 Study Design and Synthetic Cohort Definition

This study is a comparative clinical analyses of neonatal sepsis performed in high-volume neonatal intensive care units (NICUs). The study focuses on the diagnostic accuracy of flow cytometric assessment of nCD64 and nCD11b as determined by Receiver Operating Characteristic (ROC) curve analysis.[1] The population includes neonates, defined as infants aged 0 to 28 days, who were prospectively enrolled and classified into two main categories: the Sepsis Group (Case Group) and the Non-Sepsis Group (Control Group).[1]

2.2 Reference Standard Diagnosis

The definitive diagnosis of neonatal sepsis relies on positive blood culture results, which serves as the microbiological "gold standard".[7] However, given the clinical reality of culture-negative sepsis where clinical and laboratory criteria strongly suggest infection despite negative cultures the case group definition utilized highly structured inclusion criteria. This approach acknowledges that clinical sepsis diagnosis must sometimes be based on the aggregation of definitive clinical signs and conventional laboratory abnormalities.[3]

2.3 Sample Collection and Processing

Given the constraints of sampling in fragile neonatal populations, the test methodology required minimal blood volume. Samples were collected using less than 100 µl of EDTA blood, with optimal protocols requiring merely 50 µl.[4] The methodology was designed to be rapid, achieving a minimal turnaround time (TAT) ranging from 1 hour to 2 hours. This speed is essential for providing immediately actionable diagnostic results that can inform critical, time-sensitive clinical decisions.[2]

2.4 Detailed Flow Cytometry Protocol for CD64 and CD11b

The analysis of neutrophil surface markers was performed using standardized flow cytometry, typically employing a whole-blood lysis no-wash approach to minimize cell modification and processing time.[4]
Results for both nCD64 and nCD11b were quantified using two primary metrics: the percentage of neutrophils exhibiting positive expression of the marker (nCD64% and nCD11b%) and the Mean Fluorescent Intensity (MFI).[1] MFI is sometimes expressed as a standardized CD64 index.[4] Data analysis reveals that the percentage of positive cells (nCD64%) consistently demonstrates superior diagnostic performance compared to the MFI metric.[1]

2.5 Statistical Analysis

The diagnostic efficacy of the biomarkers was rigorously assessed using ROC curves to calculate the area under curve, determine optimal cutoff values, and derive measures of diagnostic utility, including sensitivity, specificity, and Predictive Values (PPV/NPV).[1] The performance of the surface markers was compared directly against established acute-phase reactants, such as high-sensitivity CRP (hs-CRP) and procalcitonin (PCT), to establish a hierarchy of diagnostic accuracy.[1]

2.6 Inclusion and Exclusion Criteria

The cohort analysis relies on rigorous inclusion and exclusion criteria designed to isolate true cases of neonatal sepsis and to establish a robust, non-infected control group.
Inclusion Criteria for the Case (Sepsis) Group
Neonates included in the case group were required to meet the following criteria:

- **Gestation:** Born at least 28 weeks gestation.
- **Maternal and Peripartum Risk Factors:** Presence of significant clinical risk factors, which include chorioamnionitis, maternal intrapartum fever, maternal urinary tract infection, prolonged premature rupture of the membrane (PROM) exceeding eighteen hours, or meco nium aspiration.
- **Composite Clinical Criteria:** In addition to risk factors, neonates had to present with at least two of the following clinical signs:
  1. Respiratory distress: Respiratory rate >60 breaths per minute, apnea (breathing pause 20 seconds.
  2. Circulatory instability: Hypotension, pallor, or a heart rate <100 beats per minute.
  3. Metabolic or Thermal abnormalities: Metabolic acidosis (ph<7.25), hypothermia (rectal temperature <36 ^{0}C or hyperthermia (> 38 ^{0}C or glucose instability (blood glucose level <45 mg/dL} or >125 mg/dL).
  4. Behavioral changes: Poor or declining activity, or feeding intolerance (increased gastric residuals >50% of milk volume in 2 feedings within 24 hours).
- **Composite Laboratory Criteria:** Patients also required at least two of the following laboratory abnormalities:
  1. Immature to total neutrophil ratio >0.2.
  2. C-reactive protein > 10mg/L
  3. White blood cell count and platelet outside the normal range

Inclusion Criteria for the Control Group
The control group comprised newborns born at or after 28 weeks gestation who were enti rely asymptomatic, lacking any sepsis-related risk factors, clinical indicators, or laboratory abnormalities. All control subjects included in comparative studies had negative blood cultures.[3]

Exclusion Criteria

Exclusion criteria were designed to eliminate confounding inflammatory states or pre-existing conditions that could skew biomarker results:

- Presence of chromosomal abnormalities.
- Existence of major congenital defects, including cardiac (congenital heart disorders), neurological (e.g., hydrocephalus), gastrointestinal (e.g., omphalocele), or renal/adrenal defects.

- Infants born before 28 weeks of pregnancy.
- Treatment with antibiotics prior to admission or prior to the initial blood sampling for the study.
- Lack of written, informed parental consent.

Results

3.1 Epidemiological Pathogen Profile and Clinical Implications

Analysis of culture data within the sepsis cohorts highlights a significant epidemiological finding: the majority of clinically defined sepsis cases (56.7%) yield negative blood cultures.[3] This high culture-negative rate underscores the vital clinical necessity for rapid and accurate surrogate biomarkers like nCD64. Since CD64 reflects the host immune response to bacterial products, it can confirm the diagnosis of cl inical sepsis, allowing for confident initiation of critical care and antibiotic protocols even when the causative organism fails to be isolated via the gold standard.[2]

Among the positive blood cultures (comprising 43.3% of cases), the most prevalent organisms identified were Gram-negative bacteria, with *Klebsiella* accounting for 26.7% of positive isolates, followed by *E. Coli* and *Staphylococcus aureus* (each at 6.7%), and *Staphylococcus Hemolyticus* (3.3%).[3] The dominance of Gram-negative pathogens provides mechanistic support for the effectiveness of CD64, as their lipopolysaccharide (LPS) components are potent activators of the innate immune cascade responsible for CD64 upregulation.

Table 1: Epidemiological Context and Pathogen Incidence in a Representative Neonatal Sepsis Cohort

Cohort Feature	Definition/Value	Citation
Culture-Negative Rate in Clinically Defined Sepsis	56.7%	3
Most Prevalent Pathogen Identified	*Klebsiella* (26.7% of positive cultures)	3
Next Most Prevalent Pathogens	*E. Coli* (6.7%), *Staph. Aureus* (6.7%)	3
Gestational Age Inclusion	geq 28 weeks	3

3.2 Comparative Diagnostic Performance of Neutrophil Markers and hs-CRP

Comparative analysis consistently demonstrates the statistical superiority of nCD64% in discriminating between septic and non-septic neonates.[1] At a determined cutoff value of 44.15%, nCD64 % achieved a sensitivity of 92.8% and a specificity of 90.8 %, resulting in a highly accurate area under the curve (AUC) of 0.894.[1] Conversely, when nCD64 was measured using Mean Fluorescent Intensity (MFI), its performance decreased significantly (Sensitivity 72.5%, Specificity 54.4%, AUC 0.634).[1]

Neutrophil CD11b demonstrated consistently poor diagnostic discrimination. nCD11b% was unable to reliably differentiate between sepsis and control groups, achieving a low sensitivity of 31.8% and an AUC of 0.405.[1] This poor performance, with an AUC value below 0.5, suggests that nCD11b may function worse than a random chance predictor in some settings, arguing strongly against its utility as a reliable standalone diagnostic tool.[1] The failure of nCD11b to perform adequately, despite its known role in activation, may be linked to rapid internalization or consumption of the marker as cells migrate into tissues, leading to inconsistent levels in the peripheral blood.[10]

For comparison, high-sensitivity CRP (hs-CRP) provided only moderate diagnostic utility, documenting a sensitivity of 69.0% and specificity of 78.15%, with a suboptimal AUC of 0.586.[1] This established a clear hierarchy of diagnostic performance, placing nCD64% statistically ahead of both nCD11b and hs-CRP as a primary biomarker.[2]

Table 2: Comparative Diagnostic Performance of Biomarkers in Neonatal Sepsis

Biomarker (Measure)	Optimal Cutoff	Sensitivity (%)	Specificity (%)	AUC	95% CI (Estimated)
nCD64 Percentage	44.15%	92.8	90.8	0.894	N/A
nCD64 MFI	1.43	72.5	54.4	0.634	N/A
nCD11b Percentage	N/A	31.8	73.6	0.405	N/A
nCD11b MFI	N/A	59.1	69.4	0.144	N/A
hs-CRP	N/A	69.0	78.15	0.586	N/A
Combined nCD64% + hs-CRP	N/A	93.9	97.2	0.938	N/A

3.3 Efficacy of Combined Biomarker Panels

To maximize diagnostic accuracy, the application of multiplexed biomarker panels has been thoroughly investigated. The synergistic combination of nCD64% and hs-CRP demonstrated the peak diagnostic performance observed in the analyzed cohorts, reaching a superior sensitivity of 93.9% and specificity of 97.2%, corresponding to an excellent AUC of 0.938.[1]

While less optimal than the combination with CRP, combining CD64 and CD11b indices (where a positive result for either marker defined a positive test) achieved a high sensitivity of 89%. However, to achieve high specificity (87\%), it was necessary for both CD64 and CD11b indices to be simultaneously positive.[11] Furthermore, research suggests that the integration of flow cytometry markers into a comprehensive sepsis score alongside other acute-phase reactants, such as PCT, can further enhance diagnostic discriminative power over any single test alone.[9]

3.4 Prognostic Performance and Severity Correlation

The utility of these markers extends beyond initial diagnosis into prognostic evaluation and monitoring of treatment response. Both CD64 and CD11b indices exhibit a statistically significant downward trend during the recovery period following successful treatment in itiation ($P < 0.01$).[11] This robust decline suggests their value as dynamic monitoring tools that could potentially guide antibiotic de-escalation by providing objective evidence that the systemic inflammatory stimulus has resolved.[11] However, the prognostic capacity of the two markers differs. nCD64 is statistically preferred over nCD11b as a diagnostic, prognostic, or monitoring sepsis marker.[2] While patients can be reclassified into severe and non-severe sepsis groups for prognostic comparisons, neither CD64 nor CD11b was found to reliably differentiate between Early-Onset Sepsis (EOS) and Late-Onset Sepsis (LOS).[2] In contrast, hs-CRP did show a significant difference between EOS and LOS groups ($p=0.04$).[2] The fact that nCD64 is a better prognostic tool than nCD11b, coupled with its highly sensitive and specific diagnostic capability, places nCD64 as the most critical flow cytometric marker for comprehensive neonatal sepsis management.[2]

4.1 **The Role of nCD64 in Enhancing Early Diagnosis and Antibiotic Stewardship**

The exceptionally high specificity and sensitivity of nCD64% establish it as a crucial biomarker for early neonatal sepsis diagnosis. With a rapid TAT of under two hours, nCD64 results can quickly confirm the presence of a robust, systemic bacterial activation state, thereby guiding immediate clinical decisions.2 The high negative predictive value (NPV) conferred by high specificity is equally critical, enabling clinicians to confidently rule out bacterial infection in symptomatic infants who are at low risk, thereby restricting the overuse of broad-spectrum antibiotics.

The mechanistic underpinning of nCD64's superior performance is rooted in its nature as the high-affinity IgG receptor. Its expression is regulated by targeted cytokine cascades, meaning its robust upregulation is a response specifically elicited by potent bacterial triggers.5 This low baseline expression and high induction capability contrast sharply with markers that are elevated across a broader spectrum of non-infectious stress or inflammation, ensuring that nCD64 provides a cleaner, more specific signal for true bacterial sepsis.

4.2 Analyzing the Diagnostic Discrepancy of nCD11b

The consistent finding that nCD11b possesses low diagnostic power highlights the complex dynamics of neutrophil adhesion molecules in peripheral circulation during sepsis. Although CD11b is known to be upregulated in systemic inflammatory states5, its utility is hampered by several factors. Its upregulation may occur in response to various non-septic stressors, significantly decreasing its specificity. More importantly, CD11b serves as an adhesion molecule mediating migration.4 Consequently, its upregulation is rapidly followed by the consumpt ion of activated neutrophils as they migrate into the focus of infection in tissues (diapedesis). This transient presence and rapid cellular consumption lead to inconsistent and unreliable levels in the peripheral blood, resulting in the poor discriminatory performance observed in diagnostic accuracy studies.10 While some specific cohort studies focusing only on preterm neonates have reported high sensitivity for nCD11b9, the aggregated evidence clearly dictates that nCD11b should not be relied upon as a primary diagnostic biomarker for general neon atal sepsis screening.2

4.3 The Synergy of Multiplexed Biomarker Panels

The finding that the combination of nCD64% and hs-CRP achieves the highest diagnostic accuracy (AUC 0.938) provides a framework for optimal clinical implementation.1 This synergy exploits the complementary temporal kinetics of the two marker types: nCD64 provides an early signal reflecting the immediate innate immune activation (cellular marker), while hs-CRP reflects the subsequent, typically slower, hepatic acute-phase response (humoral marker). Using these markers together ensures that the diagnostic window is maximized, covering both the initial cytokine storm phase and the established systemic inflammation phase. Ultimately, to maximize predictive value, nCD64 should be incorporated into a comprehensive, weighted sepsis score alongside PCT, CRP, and hematological indices, thereby overcoming the drawbacks of each test used in isolation.12

4.4 Epidemiological and Clinical Correlation

The prevalence of Gram-negative organisms, such as Klebsiella and E. Coli, in the study cohorts3 aligns with the robust upregulation of CD64, as these pathogens are potent inducers of the requisite inflammatory pathways.

The clinical reality that over half of the documented clinical sepsis cases are culture-negative3 solidifies the role of nCD64 as an essential tool. In these common clinical scenarios, waiting for a definitive culture result is impossible, and conventional markers are insuf ficiently accurate. CD64 provides rapid, objective proof of a severe systemic bacterial response, enabling clinicians to continue antibiotic regimens with confidence, thereby preventing delays that could lead to lifelong morbidities or mortality.1 Moreover, the observation that nCD64 levels decrease significantly during the recovery period suggests a high potential for its use in dynamic monitoring, allowing for better-informed decisions regarding the duration of antibiotic therapy.11 While CD64 fails to distinguish between EOS and LOS, its value lies in its strong correlation with the overall inflammatory burden, marking it as a superior monitoring tool than CD11b for assessing treatment efficacy and severity stratification.2

4.5 Translational Barriers and Future Directions

Despite the established diagnostic superiority of nCD64, its routine, multicentric adoption is currently hindered by the lack of global standardization in flow cytometry protocols.4 Variations in staining, gating strategies, lysis systems, and instrument calibr ation render cross-study comparisons difficult. Future translational research must prioritize the establishment of standardized protocols for au tomated analysis, specifically focusing on the percentage of positive neutrophils (nCD64%) rather than MFI, to ensure comparable and reliable c ut-off values across different NICUs and laboratories.4 Furthermore, while nCD64 is established as a better prognostic marker than nCD11b2, definitive research is needed to quantify specific prognostic cutoffs that correlate with severe sepsis, length of hospital stay, and mortality, thereby fully integrating this biomarker into severity stratification guidelines.6

Conclusion

Neutrophil CD64, quantified as the percentage of positive cells (nCD64%), is a highly accurate, sensitive, and rapid diagnost ic modality for neonatal sepsis. Its robust performance (AUC 0.894) statistically surpasses that of nCD11b (AUC 0.405) and hs-CRP (AUC 0.586) as a standalone marker. Optimal diagnostic efficacy (AUC 0.938) is achieved by combining nCD64% with hs-CRP, utilizing their distinct yet complementary temporal kinetics. The feasibility of measuring nCD64 within one to two hours using minimal blood volume positions it as an ideal test for routine application in the NICU, significantly aiding in the prompt diagnosis of both culture-positive and culture-negative clinical sepsis and facilitating judicious antibiotic stewardship. To realize its full potential, global efforts must focus on standardizing flow cytometry protocols and establishing universally accepted quantitative cut-off values.

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