Original Article

Microbial Spectrum in Adult Sepsis and Its Correlation with Neutrophil CD64 (nCD64) Expression: A Cross-Sectional Study at a Tertiary Care Centre

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Abstract

Background: Sepsis is a life-threatening condition caused by a dysregulated host response to infection, with significant morbidity and mortality rates worldwide. Early identification of causative microorganisms and biomarkers is critical in managing sepsis. Methicillin-resistant Staphylococcus aureus (MRSA) is known to be the most common, with a global pooled prevalence of 14.69%. This cross-sectional study evaluates the microbial spectrum in adult sepsis and the correlation with neutrophil CD64 (nCD64) expression.

Materials and Methods: This cross-sectional study was conducted over 18 months and included 94 adult patients clinically suspected of sepsis at a tertiary care center in Vijayapura. Blood samples were analyzed for nCD64 expression using flow cytometry, and blood culture sensitivity tests were performed. Statistical analysis was used to assess the correlation between microbial isolates and CD64 expression levels.

Results: Of the 94 patients, 62.8% had positive cultures, with MRSA (27.1%) and Klebsiella pneumoniae (20.3%) being the most prevalent. nCD64 expression was significantly higher in culture-positive cases, particularly in Gram-negative infections ($p = 0.001$). These findings highlight the utility of nCD64 as a diagnostic and prognostic marker in managing sepsis.

Conclusion: The study demonstrates a strong correlation between neutrophil CD64 expression and microbial isolates in adult sepsis patients, particularly with Gram-negative microorganisms. Higher nCD64 expression levels are indicative of bacterial infections, especially Gram-negative bacterial infections, thus making it a useful diagnostic biomarker in sepsis detection.

Keywords:

flow cytometry, microbe, bacteria, neutrophils, CD64, sepsis

Introduction

Sepsis remains a significant healthcare challenge worldwide, contributing to high morbidity and mortality rates, particularly in critically ill patients. The microbial spectrum in sepsis is diverse, ranging from Gram-positive and Gram-negative bacteria to fungi, viruses, and, occasionally, parasites. It has been found that the pooled prevalence of bacterial strains like Methicillin-Resistant Staphylococcus aureus (MRSA) worldwide is approximately 14.69% [1], while the overall burden of sepsis globally was around 48.9 million cases as of 2020, resulting in over 11 million sepsis-related deaths [2]. Therefore, understanding the

causative microorganisms and their pathophysiological impact is crucial for early diagnosis and management. In adult sepsis, prompt identification of the causative agents and their virulence factors can significantly influence patient outcomes, including survival and recovery rates [3].

Neutrophils, as the first responders to infection, are central to this response. Among the various markers associated with neutrophil activity, CD64 (Fc-gamma receptor 1) has gained increasing attention as a promising biomarker. CD64 is a high-affinity receptor for the Fc region of IgG, and its expression on neutrophils is significantly upregulated during infection, inflammation, and sepsis [4].

Numerous studies have highlighted neutrophil CD64 expression as an early indicator of bacterial infections, with its levels correlating well with the severity of sepsis [4, 5]. CD64 expression has shown promise as a marker for distinguishing bacterial sepsis from other causes of systemic inflammation and non-infectious conditions, thus aiding in early diagnosis and targeted therapy [4]. The correlation between the microbial spectrum and neutrophil CD64 expression may provide insights into the host's immune response to different pathogens [3, 5]. Furthermore, CD64 expression is often proportional to the severity of infection, suggesting that its quantification can be a useful tool for assessing disease progression and prognosis [6].

While nCD64 has been explored in global studies, limited research has focused on its correlation with specific microbial patterns in the Indian clinical setting. This study seeks to fill this gap by evaluating the microbial spectrum of sepsis in adult patients and correlating it with nCD64 expression, wherein lies its novelty. The aim is to provide insights that can enhance diagnostic workflows, improve early detection, and guide targeted interventions for better patient outcomes.

The objective of this study is to evaluate the microbial spectrum responsible for adult sepsis and to correlate it with nCD64 expression in order to assess its potential as a diagnostic marker.

Materials and Methods

Study Design: A cross-sectional study was carried out over a period of 18 months, from September 1, 2022, to February 29, 2024, involving 94 adult patients at Shri B.M. Patil Medical College, Hospital, and Research Centre, BLDE (Deemed to be University), Vijayapura, Karnataka, India. The study commenced after receiving ethical clearance from the Institutional Ethical Committee of BLDE (Deemed to be University) on August 30, 2022.

Study Population: Patients were enrolled in the study based on clinical signs suggestive of sepsis, using qSOFA scoring criteria. Patients with a history of hematological malignancies, diabetes mellitus, rheumatoid arthritis, or chronic liver disease were excluded.

Methods of Data Collection: The study analyzed neutrophil CD64 (nCD64) expression and blood culture sensitivity tests. Upon admission, all patients suspected of sepsis underwent evaluation for nCD64 expression through flow cytometry, along with blood culture sensitivity testing, as well as general and systemic examinations. Blood samples were collected in ethylenediaminetetraacetic acid (EDTA) tubes to prevent clotting and ensure accurate measurement of CD64 levels. These levels were assessed using the BD FACSLyric™ flow cytometer (BD, Franklin Lakes, NJ) within 24 hours of collection.

Blood cultures can have low sensitivity, particularly in patients who have received prior antibiotic treatment, leading to false negatives. They are also time-consuming, often taking 24–72 hours for results, delaying diagnosis and treatment. Additionally, contamination risks can produce false-positive results.

Antibody Titration: Two milliliters (mL) of freshly collected human peripheral blood treated with EDTA anticoagulant was used. The total staining volume and the number of cells for the multicolor experiment were calculated, with an estimated one million cells in 100 µL, combined with 50 µL of the antibody cocktail, giving a total staining volume of 150 µL. Serial dilutions of the CD64 antibody were performed to ensure precise quantification of nCD64 expression via flow cytometry. This method allowed for distinguishing between dim, moderate, and bright expression levels, which is crucial for diagnosing sepsis. The recommended antibody serum volume was 20 µL per test.

Stain buffer was added to the tubes as outlined in Table 1 (50 µL in each tube, except Tube 1). Next, 40 µL of CD64 allophycocyanin antibody was added to Tube 1, mixed thoroughly, and then 50 µL was aspirated from Tube 1 and transferred to Tube 2. This procedure was repeated for subsequent tubes (as shown in Table 1). Tube 7 served as the unstained control with no antibody added. The stain-lyse-wash surface staining protocol was followed for sample preparation.

Table 1: CD64 antibody titration procedure.

	Stain Buffer	Transfer from previous tube	Temporary volume	Transfer to next tube	Final Ab – mix volume	Ab (µL)
Tube 1	60µL	40µL Ab	100µL	50µL	50µL	20.00
Tube 2	50µL	50µL	100µL	50µL	50µL	10.00
Tube 3	50µL	50µL	100µL	50µL	50µL	5.00
Tube 4	50µL	50µL	100µL	50µL	50µL	2.50
Tube 5	50µL	50µL	100µL	50µL	50µL	1.25
Tube 6	50µL	50µL	100µL	50µL	50µL	0.6250
Tube 7	50µL	0µL	50µL	NA	50µL	0.0000

Granulocytes, including neutrophils, were identified by their distinctive forward scatter and side scatter characteristics compared to other leukocytes, and a manual gate was drawn around this population. The granulocytes were then further analyzed by generating a new plot from the previously gated group. A final gate was applied specifically to the neutrophils based on their scatter properties. Data analysis was conducted, including the plotting of signal-to-noise ratios, and the mean fluorescence intensity (MFI) of nCD64 was measured. MFI represents the average intensity of the fluorescent signal emitted by stained neutrophils, indicating the expression level of CD64 on their surface.

Statistical Analysis: The data were entered into a Microsoft Excel sheet (Microsoft Corp., Redmond, WA) and analyzed using SPSS Version 20 (IBM Corp., Armonk, NY). Neutrophil CD64 (nCD64) levels were compared between culture-negative patients to assess the degree of expression. Pearson's correlation was utilized for analysis, and statistical significance was established at a p-value < 0.05.

Results

The ages of participants in this study ranged from 19 to 87 years, with a mean age of 48 ± 18 years. Among the 94 patients, 29 (30.9%) were aged between 19–35 years, 25 (25.5%) were between 36–50 years, another 25 (25.5%) were in the 51–65-year group, and 17 (18.1%) were over 65 years old (Figure 1).

Among the total number of patients in this study, 45 (47.9%) were males, and 49 (52.1%) were females (Figure 2).

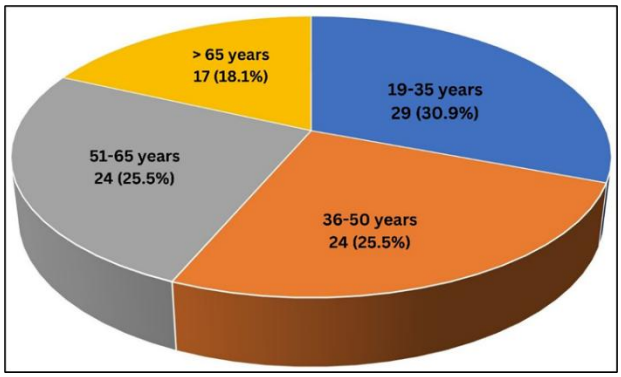

Figure 1: Distribution of patients according to different age groups. Blue represents age group of 19-35 years; Orange represents age group of 36-50 years; Grey represents age group of 51-65 years; Yellow represents age group of >65 years.

The blood culture test was positive in 59 (62.8%) of 94 cases and negative in 35 (37.2%). All 94 samples were classified into dim, moderate, and high nCD64 expression categories based on scattergram intensity. Qualitative analysis of nCD64 expression showed bright expression in 39 patients (41.5%), dim expression in 35 patients (37.2%), and moderate expression in 20 patients (21.3%), as depicted in Figure 3.

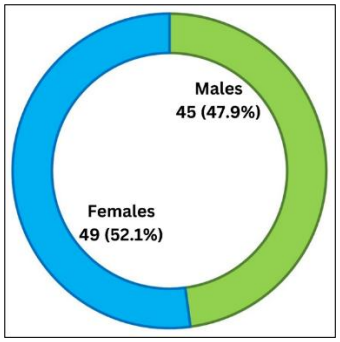

Figure 2: Distribution of patients according to gender. Blue represents females, which comprise 49 (52.1%) of the 94 cases; Green represents males, which comprises of the remaining 45 (47.9%) cases.

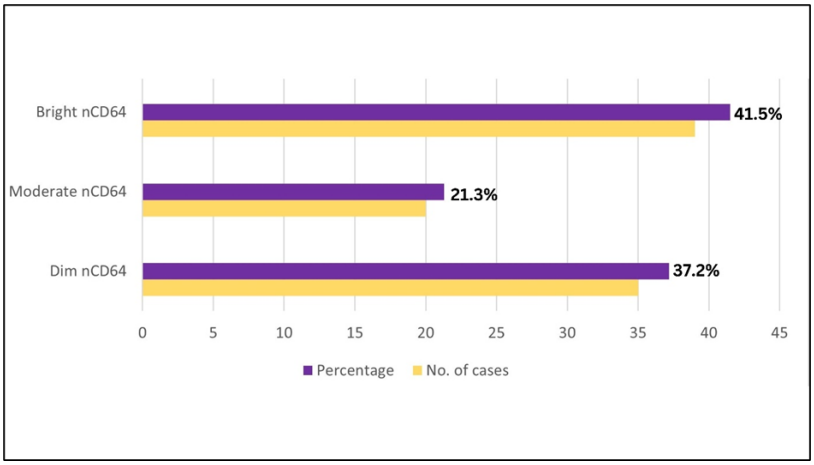

Figure 3: Expression of nCD64 categorized as dim, moderate, and bright. The x-axis represents the no. of cases; y-axis represents nCD64 expression.

The organisms isolated through culture among the sepsis-positive cases were found to be of seven types, including Methicillin-resistant Staphylococcus aureus (MRSA), Klebsiella pneumoniae spp. pneumoniae, Pseudomonas aeruginosa, Staphylococcus epidermidis, Staphylococcus hominis, Escherichia coli (E. coli), and Acinetobacter baumannii complex. Out of these seven organisms, the predominant ones were Methicillin-resistant Staphylococcus aureus, which was isolated in 16 (27.1%) cases, and Klebsiella pneumoniae spp. pneumoniae, which was isolated in 12 cases (20.3%). These two organisms accounted for 28 (47.4%) of the total number of sepsis-positive patients, i.e., 59 cases. These organisms were followed by Pseudomonas aeruginosa, which was isolated in 11 cases (18.7%), and Staphylococcus epidermidis, which was isolated in 8 cases (13.5%). This distribution has been summarized in Table 2 below.

Therefore, in the present study, the four organisms Methicillin-resistant Staphylococcus aureus (MRSA), Klebsiella pneumoniae spp. pneumoniae, Pseudomonas aeruginosa, and Staphylococcus epidermidis were the majority, i.e., they comprised 47 (80%) out of the 59 culture-positive cases. This indicates that these organisms are responsible for the majority of sepsis cases within the present study setting (Figure 4).

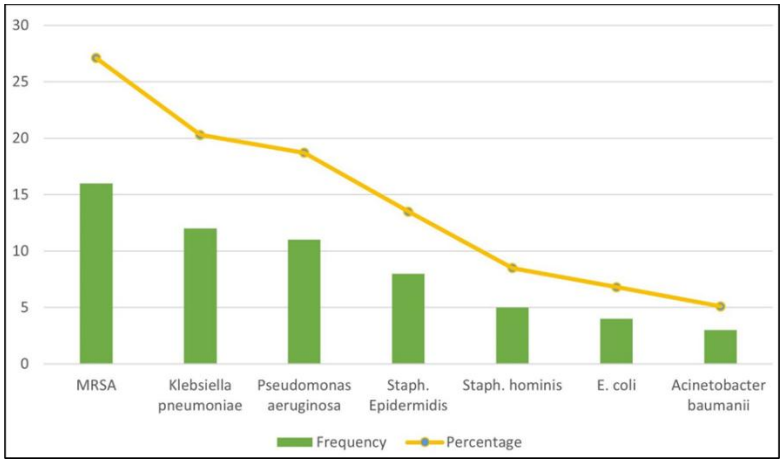

Figure 4: Prevalence of the different types of isolated microorganisms among the confirmed sepsis cases. x-axis represents Name of the organism; y-axis represents No. of cases.

Table 2: Prevalence and Distribution of Microorganisms in Sepsis Patients

Organism Isolated	Frequency (%)
Methicillin Resistant Staphylococcus aureus	16 (27.1)
Klebsiella pneumoniae spp. pneumoniae	12 (20.3)
Pseudomonas aeruginosa	11 (18.7)
Staphylococcus epidermidis	08 (13.5)
Staphylococcus hominis	05 (8.5)
Escherichia coli	04 (6.8)
Acinetobacter baumanii complex	03 (5.1)
Total	59 (100.0)

All the culture-positive cases constituted organisms strictly of bacterial origin. The isolated organisms were further classified as gram-positive and gram-negative. Methicillin-resistant Staphylococcus aureus (MRSA), Staphylococcus epidermidis, and Staphylococcus hominis constituted gram-positive organisms, whereas Klebsiella pneumoniae, Pseudomonas aeruginosa, E. coli, and Acinetobacter baumannii constituted gram-negative organisms in our study.

Correlation of nCD64 with the nature of the isolated organism: Out of 39 patients who had bright nCD64 expression, 19 (48.7%) were found to have gram-negative organisms, and 20 (51.3%) had gram-positive organisms in their blood samples. Out of 20 patients who had medium nCD64 expression, 10 (50%) had gram-negative organisms, 8 (40%) had gram-positive organisms, whereas 2 (10%) had negative blood cultures. Out of 35 patients who had dim nCD64 expression, 1 (2.9%) had a gram-negative organism, 1 (2.9%) had a gram-positive organism, and blood culture was negative in 33 (94.3%) patients. The correlation between nCD64 expression and the organism isolated was statistically significant (p = 0.001), as represented in Table 3 below.

Table 3: Correlation of nCD64 expression with the nature of isolated organisms; Sterile: Blood culture negative; * represents significant p-value (< 0.05)

Organism Isolated		**nCD64 Expression**			**Total**
		Bright	**Medium**	**Dim**	
Gram Negative	Count	19	10	1	1
	%	48.7%	50.0%	2.9%	1.1%
Gram Positive	Count	20	8	1	15
	%	51.3%	40.0%	2.9%	16.0%
Sterile	Count	0	2	33	78
	%	0.0%	10.0%	94.3%	83.0%
Total	Count	39	20	35	94
	%	100.0%	100.0%	100.0%	100.0%
Chi-square value- 78.57					
p value- 0.001*					

Discussion

This study confirms the utility of neutrophil CD64 (nCD64) expression as a diagnostic biomarker for sepsis, particularly in distinguishing between Gram-positive and Gram-negative bacterial infections. Our results align with existing literature, such as the findings of Wang et al. [7], who also reported elevated nCD64 expression in Gram-negative bacterial sepsis. In our study, bright nCD64 expression was observed in 41.5% of the patients, and a statistically significant correlation was found between high nCD64 expression and Gram-negative infections (p = 0.001). Similarly, Ghosh et al. [8] highlighted that 60% of Gram-negative infections exhibited elevated nCD64 expression, reinforcing the findings of our study.

In comparison to other biomarkers, such as C-reactive protein (CRP) or procalcitonin (PCT), nCD64 has shown superior sensitivity and specificity. Cardelli et al. [9] demonstrated that nCD64 expression had 96% sensitivity and 95% specificity for detecting bacterial sepsis, particularly in Gram-negative bacteremia. This aligns closely with our findings. Additionally, Chang et al. [10] and Farias et al. [11] corroborated that nCD64 expression can differentiate between bacterial and non-bacterial infections, making it a reliable tool for guiding clinical decision-making. In contrast, CRP and PCT levels may rise in various non-infectious inflammatory conditions, potentially leading to false positives.

More recent studies conducted by Patnaik et al. [12] and Hu et al. [13] reported that nCD64 expression was effective in distinguishing between Gram-positive and Gram-negative sepsis, with higher expression seen in Gram-negative cases, which was, again, in line with the present study.

Gibot et al. [14], Jalava-Karvinen et al. [15], Hsu et al. [16], and Gerrits et al. [17] also demonstrated that nCD64 had high sensitivity and specificity in identifying bacterial sepsis, particularly in Gram-negative cases. Therefore, the consistent findings

across these studies and our own highlight the diagnostic value of nCD64 expression in sepsis, particularly in differentiating between Gram-positive and Gram-negative bacterial infections.

Furthermore, nCD64 has potential clinical applications beyond diagnosis. Sequential monitoring of its expression can help track disease progression and evaluate the effectiveness of therapeutic interventions. Gram-negative infections are often associated with more severe disease courses. Assessing nCD64 can therefore guide clinicians in prioritizing interventions for high-risk patients. By integrating nCD64 measurement into existing diagnostic workflows, clinicians can overcome limitations associated with blood cultures, such as low sensitivity and prolonged turnaround times.

Potential Biases and Study Limitations: One significant limitation of our study is the relatively small sample size (n = 94), which may affect the generalizability of the findings. Larger, multicenter studies would provide more robust data, ensuring that results are representative of diverse populations and bacterial spectra. Furthermore, patient selection bias may exist, as this study was conducted at a single tertiary care center. Patients at such facilities are often more critically ill than those in general hospitals, which may skew the results toward higher nCD64 expression levels. Another limitation lies in the reliance on blood culture as the gold standard for detecting microbial infections. While blood cultures are widely accepted, they are known for their limited sensitivity, especially in patients pre-treated with antibiotics.

Conclusion

This study underscores the critical role of nCD64 as a diagnostic and prognostic marker in sepsis management. By correlating nCD64 expression with microbial isolates, particularly Gram-negative bacteria, it demonstrates the biomarker's potential to enhance early detection and guide targeted therapy. The findings highlight the limitations of conventional diagnostic methods, such as blood cultures, and advocate for the incorporation of nCD64 measurement into routine clinical practice. The use of nCD64 can transform sepsis management by enabling faster diagnosis, improving risk stratification, and facilitating better therapeutic decisions. For instance, patients with high nCD64 expression levels, particularly those with Gram-negative infections, can be prioritized for aggressive interventions. Future research should focus on multicenter studies with larger cohorts to validate these findings and explore the cost-effectiveness of implementing nCD64 testing on a broader scale. Integrating nCD64 into diagnostic workflows could significantly reduce diagnostic delays, improve patient outcomes, and alleviate the burden of sepsis in healthcare systems worldwide.

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Statement of human and animal rights: *All authors have confirmed that the procedures followed were in accordance with the*

ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 (5). All authors have confirmed that this study did not involve animal subjects or tissue.

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