

# Role of CD-64 on Neutrophils and HLA-DR on Monocytes as Markers of Neonatal Sepsis: A Cross-sectional Study

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## ABSTRACT

**Introduction:** Neonatal sepsis remains a diagnostic burden globally, responsible for about 30-50% of the total neonatal deaths each year in developing countries. Neutrophil CD-64 is found to be a promising marker for the diagnosis of early and late infections in newborns. Human Leukocyte Antigen-DR (HLA-DR) is a glycosylated cell surface transmembrane protein expressed on monocytes, allowing antigen presentation to T-cells and playing a crucial role in initiating the immune cascade during sepsis. Decreased expression of HLA-DR on monocytes has been associated with decreased survival in newborns with sepsis.

**Aim:** To evaluate the role of neutrophil CD-64 and monocyte HLA-DR expression as markers of neonatal sepsis.

**Materials and Methods:** This was a cross-sectional prospective study carried out at the Postgraduate Institute of Medical Sciences, Rohtak, Haryana, India, between July 2016 and June 2017. Total of 70 full-term neonates with clinical suspicion of sepsis were enrolled. A 2 mL peripheral venous blood sample was collected for flow cytometry, blood culture, and sepsis screening in all patients. The expression of cell surface markers (CD-64 on neutrophils and HLA-DR on monocytes) was measured by an eight-color flow cytometer. A composite parameter was derived by dividing the Mean Fluorescence Intensity (MFI) values of nCD-64 and their respective mHLA-DR, multiplying the ratio by 100, and terming it as the sepsis index (Sepsis Index = nCD-64/mHLA-DR x 100). A region was drawn on monocytes on an

SSC/CD14 plot. Gating was performed on 'not monocytes' on the SSC/CD45 bivariate dot plot, and regions were drawn on lymphocytes and neutrophils. Data were entered into a Microsoft Excel spreadsheet and analysed using Statistical Package for Social Sciences (SPSS) version 21.0 statistical software. The Chi-square test was applied for proportions, and the Analysis of Variance (ANOVA) test was applied for normally distributed data.

**Results:** In this study, 70 symptomatic neonates clinically suspected to have sepsis were enrolled and categorised into the sepsis group and the no Sepsis group. The sepsis group was further subgrouped into Definite Sepsis (Blood culture positive) and Probable Sepsis (Symptomatic baby with sepsis screen positive but sterile blood culture). nCD-64 positivity was observed in all cases (n=19) of definite sepsis. nCD-64 revealed 100% sensitivity, 87.5% specificity, 86.36% Positive Predictive Value (PPV), 100% Negative Predictive Value (NPV), and 93.02% diagnostic accuracy in culture-positive sepsis. However, downregulation of mHLA-DR observed in the present study alone showed poor diagnostic utility. The Sepsis index showed sensitivity of 94.73%, specificity of 62.50%, PPV of 66.66%, NPV of 93.75%, and accuracy of 76.74% in the definite sepsis group.

**Conclusion:** Flow cytometric assessment of neutrophil CD-64 may be considered a rapid and reliable marker for the diagnosis of bacterial neonatal sepsis. mHLA-DR may be beneficial for monitoring patients at a later point in time for the identification of delayed immuno-suppression in neonatal sepsis.

**Keywords:** Neutrophil CD-64, Monocyte HLA-DR, Sepsis Index, Flowcytometry

## INTRODUCTION

Neonatal sepsis remains a diagnostic burden globally, responsible for about 30-50% of the total neonatal deaths each year in developing countries. It is estimated that up to 20% of neonates develop sepsis, and approximately 1% die of sepsis-related causes [1]. At Neonatal Intensive Care Units (NICU), empirical antibiotic therapy is commonly initiated for infants with suspected sepsis. Un-necessary administration of antibiotics leads to an increase in multi-resistant germs, as well as costs and the risk of related adverse effects. Many studies attempt to correlate clinical and laboratory findings with the presence of proven sepsis. To date, none of them have managed to define the most adequate parameters to diagnose neonatal sepsis with certainty [2].

The Total Leucocyte Count (TLC), Absolute Neutrophil Count (ANC), immature to total neutrophils ratio (I:T), platelet count, C-Reactive Protein (CRP), and micro ESR (m-ESR) are used as screening tools, but these tests are less sensitive and specific [3]. Blood culture is still considered the 'gold standard' for the diagnosis of septicaemia; however, blood culture takes 48-72 hours for the results to be available [4]. Additionally, negative blood cultures do not exclude the presence of neonatal sepsis, which is why other tests in the diagnosis of neonatal sepsis are warranted [5].

Recently, numerous cell surface antigens have been studied as promising biomarkers of infection, including CD11b, CD-64, CD-69, and HLA-DR [6]. Flow cytometric analysis has the advantage over conventional haematological and immunological assay methods by being able to localise activated markers to a specific cell type.

Neutrophil CD-64 is found to be a promising marker for the diagnosis of early and late infections in newborns [7]. The CD-64, known as Fc-gamma receptor 1 (FcγRI), binds monomeric IgG-type antibodies with high affinity in the process of phagocytosis and intracellular killing of opsonised microbes. It is expressed on antigen-presenting cells (monocytes, macrophages, and dendritic cells) and only weakly on resting neutrophils. During neutrophil activation, under the influence of inflammatory cytokines, there is an upregulation of neutrophil CD-64, which is considered to be a very early step in the host's immune response to bacterial infection. Importantly, neutrophils from preterm infants express CD-64 during bacterial infections to the same degree as those from term infants, children, and adults [8].

HLA-DR is a glycosylated cell surface transmembrane protein expressed on antigen-presenting cells and constitutively expressed on monocytes. HLA-DR allows antigen presentation to T cells and is crucial for the initiation of the immune cascade during

sepsis. Decreased expression of HLA-DR on monocytes has been associated with decreased survival in newborns with sepsis; however, the mechanism for this reduced surface expression of HLA-DR has not been established [9-12].

Although many infection markers have been evaluated in the neonatal intensive care setting, none is ideal. Leukocyte cell surface antigens have the potential to serve as infection markers in clinical practice. Rapid and objective assessment of their expression on leukocytes makes them attractive for consideration as potential diagnostic markers of neonatal sepsis [9]. Thus, there is great enthusiasm in studying diagnostic markers that can aid in early distinction between infected and non-infected infants.

Therefore, the objective of this study was to evaluate the role of neutrophil CD-64 and monocyte HLA-DR expression as markers of neonatal sepsis.

## MATERIALS AND METHODS

This was a cross-sectional study carried out in the Department of Pathology in collaboration with the Neonatal services division, Department of Paediatrics, Postgraduate Institute of Medical Sciences, Rohtak, Haryana, India between July 2016 and June 2017. The study was approved by the Institutional Ethical Committee of University of Health Sciences (IEC-15/498,24/03/15). Written informed consent was obtained from the parents or guardians for all study patients.

**Inclusion criteria:** Total of 70 consecutive full-term ( $\geq 37$  weeks of gestation), Appropriate for Gestational Age (AGA) neonates (aged 0-28 days) with clinical suspicion of sepsis and requiring antibiotic therapy were enrolled, provided they had not received antibiotics in the preceding 72 hours.

The signs and symptoms included unstable temperature ( $>36.5^{\circ}\text{C}$  or  $>37.5^{\circ}\text{C}$  on two occasions within 12 hours), respiratory distress as evidenced by tachypnoea (respiratory rate  $>60$  breaths/min), intercostal or sub-sternal retractions, apnoea, central cyanosis, increase in anterior fontanelle tension or convulsion, persistent vomiting, bloody stool, and abdominal distension. A full sepsis screen was performed.

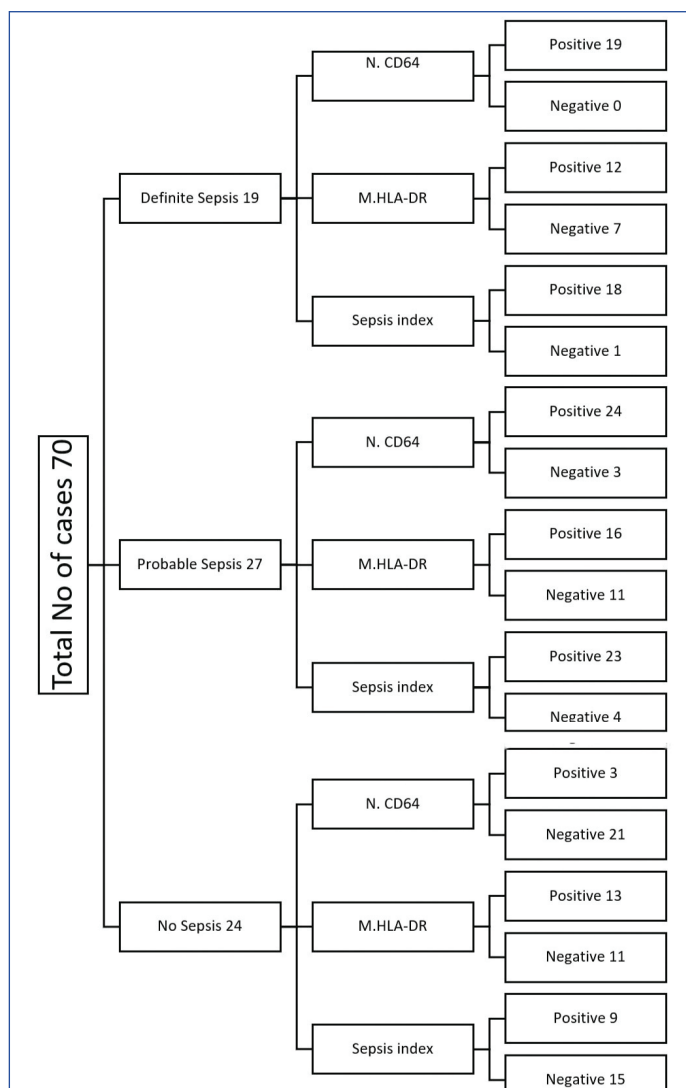
**Exclusion criteria:** Neonates with major congenital malformations, severe birth asphyxia (APGAR score  $<3$  at 5 minutes or cord pH less than 7), and those who received antibiotics in the preceding 72 hours were excluded.

## Procedure

Neonatal sepsis was categorised as early-onset neonatal sepsis (EONS, within the 'first' 72 hours of life) and late-onset neonatal sepsis (LONS, after the 'first' 72 hours of life).

In this study, 70 symptomatic neonates clinically suspected of having sepsis were enrolled and categorised into the Sepsis group and No Sepsis group. The sepsis group was further sub-grouped into Definite Sepsis (Blood culture positive) and Probable Sepsis (Symptomatic baby with sepsis screen positive but sterile blood culture). Cases that failed to meet the criteria of the sepsis group were categorised as the 'No Sepsis group' (Sepsis screen negative and blood culture sterile) [Table/Fig-1].

Among 70 neonates, 30 age matched healthy full-term, AGA neonates without major congenital malformations, severe birth asphyxia and clinical suspicion of sepsis were taken as controls. A 2 mL peripheral venous blood sample was collected for flow cytometry, blood culture, and sepsis screening in all patients after a detailed history, physical examination, and recording of clinical signs of sepsis, just before the start of antibiotics. The sepsis screen included C-REACTIVE PROTEIN (CRP), Total Leukocyte Count (TLC), Absolute Neutrophil Count (ANC), immature to total neutrophil ratio (I:T), and micro Erythrocyte Sedimentation Rate (mESR).



[Table/Fig-1]: Study flowchart.

Complete blood count was estimated by an automated hematology Analyser (Mindray BC5800) and then re-checked manually by a peripheral blood film. The TLC of less than 5000/mm<sup>3</sup> and I/T ratio of more than 0.2 were considered abnormal. The ANC was considered abnormal if the value fell outside the limits of normalcy as per the charts of Manroe BL et al., [13]. The micro ESR was considered positive if it was above age in days +3mm in 1<sup>st</sup> hour in the first week of life or greater than 10 mm in the 1<sup>st</sup> hour thereafter. CRP was done by the qualitative method of latex agglutination in a dilution of 1:1 and considered positive at a level of 1.2 mg/dL. Blood culture was done by the aerobic method as per standard microbiological protocols- Clinical and Laboratory Standards Institute guidelines [14]. The sepsis screen was considered positive if two parameters out of five were positive.

**Flow cytometric analysis:** Cell surface markers (CD-64 on neutrophils and HLA-DR on monocytes) expression was measured by an eight-color Flow cytometer BD FACS Canto II (Becton Dickinson, San Jose, CA) system. All analyses and interpretations were carried out using the FACS-Diva software (BD Biosciences). Ethylene Diamine Tetra-acetic Acid (EDTA) anti-coagulated peripheral blood samples at the time of initial presentation were collected, and all the samples were processed within 12 hours. Samples were stored at 2-8°C between the time of collection and processing. The Stain-Lyse-wash method was used for the preparation of samples. A 50  $\mu\text{L}$  of whole blood was taken in the appropriately labeled 12 $\times$ 75 mm Fluorescence-Activated Cell Sorting (FACS) tube. CD14 Fluorescein Isothiocyanate (FITC) conjugated 10  $\mu\text{L}$ , CD-64/CD-45 (Quantibrite) PE/PerCP conjugated 10  $\mu\text{L}$ , and HLA-DR APC conjugated 3  $\mu\text{L}$  antibodies were used. After preparation, samples were run on a pre-calibrated flow cytometer.

**Acquisition:** Forward Scatter (FSC) and Side Scatter (SSC) were

on linear amplification, and fluorescent channels were on log amplification. The threshold was set on CD-45 PerCP to include Lymphocytes, Monocytes, and Neutrophils. The gate was stopped at 30,000 all events. All events were stored.

**Analysis:** A region was drawn on monocytes on a SSC/CD-14 plot. Gating was done on 'not monocytes' on SSC/CD-45 bivariate dot plot, and regions were drawn on lymphocytes and neutrophils.

**Interpretation:** Neutrophilic CD-64 was designated as nCD-64 and monocytic HLA-DR as mHLA-DR. To combine changes in the expression of pro-inflammatory (nCD-64) and anti-inflammatory (mHLA-DR) markers, authors evaluated a parameter by dividing the Median Fluorescence Intensity (MFI) values of nCD-64 and their respective mHLA-DR and multiplying the ratio by 100, termed as the sepsis index.

Sepsis Index= nCD-64/mHLA-DR x 100 [15].

The cut-off value of positivity for parameters CD-64 and Sepsis index which were upregulated was derived by Mean+2 SD (standard deviation) after running 30 samples of healthy neonate, and the value below the 10<sup>th</sup> percentile of the healthy controls was taken as the cut-off value for the parameters which were down-regulated (mHLA DR). However, Receiver Operator Characteristics curve (ROC) was also applied to find the optimal cut-off values. The values were almost the same by both methods. The cut-off value derived for nCD-64 was 2001.24 (MFI), for mHLA DR was 8218 (MFI), and for the Sepsis index was 19.18.

## STATISTICAL ANALYSIS

Data was entered into a Microsoft excel spreadsheet and doubly checked for errors. Data was coded appropriately by the investigator. Data were analysed using Statistical Package for Social Sciences (SPSS) version 21.0 statistical software. Chi-square test was applied for proportions, and Analysis Of Variance (ANOVA) test was applied for normally distributed data. Data were considered significant if the p-value was <0.05. Diagnostic statistics like sensitivity, specificity, PPV, NPV, and accuracy were calculated based on the cut-off values of various parameters.

## RESULTS

In this study, 70 symptomatic neonates, clinically suspected to have sepsis, were enrolled and categorised into Sepsis group and No Sepsis group. Thirty age-matched healthy full-term neonates without clinical suspicion of sepsis were taken as controls. The sepsis group was further subgrouped into Definite Sepsis (Blood culture positive) and Probable Sepsis (Symptomatic baby with sepsis screen positive but sterile blood culture). The cases that failed to meet the criteria of the sepsis group were categorised as 'No Sepsis group' (Sepsis screen negative and blood culture sterile). Neonates in the 'No Sepsis' group initially presented with signs and symptoms of sepsis. However, their septic screen was negative, and blood culture was sterile. Therefore, they were categorised as the 'No Sepsis' group. The most common bacteria isolated in blood culture-proven definitive sepsis were *Staphylococcus aureus* followed by coagulase-negative *Staphylococcus*. The EONS group constituted 54.29% (38/70) of the total cases, whereas the LONS group constituted 45.71% (32/70).

[Table/Fig-2] illustrates the demographic parameters of the study population (cases) and control. Out of the 70 neonates studied, there were 39 males and 31 females.

The incidence of premature rupture of membranes was higher in the infected group compared to the non-infected group, 36.95% (17/46) versus 16.67% (4/24). All other characteristics were comparable between all groups. [Table/Fig-3] shows the various laboratory parameters in the study population. The micro ESR was considered positive if it was above age in days +3 mm in 1<sup>st</sup> hour" in the first week of life or greater than 10 mm in hour thereafter. The laboratory parameters were not comparable between the sepsis group and

Parameter	Cases (N=70)			Healthy control (n=30)
	Definite sepsis (n=19)	Probable sepsis (n=27)	No sepsis (n=24)	
Gestational age in days {Mean (±SD)}	268.1 (±6.4)	270.8 (±6.3)	267.8 (±5.2)	270.1 (±5.1)
Birth weight in grams {Mean (±SD)}	2805.2 (±153.9)	2825.9 (±237.9)	2875.0 (±189.3)	2883.3 (±136.6)
Age <3 days (EONS)	9 (23.6%)	12 (31.5%)	17 (44.7%)	18 (60.0%)
Age >3 days (LONS)	10 (31.2%)	15 (46.8%)	7 (21.8%)	12 (40.0%)
Meconium-stained liquor	7 (36.8%)	6 (22.2%)	6 (25.0%)	0.0
PROM*	6 (31.5%)	11 (40.7%)	4 (16.6%)	0.0

**[Table/Fig-2]:** Demographic Characteristics of the study population (Cases=70, Control=30).

\*PROM: Premature rupture of membranes.

the no sepsis group. It was observed that the incidence of various laboratory parameters like abnormal TLC, ANC, I:T ratio, and platelet count was significantly higher in the sepsis group compared to the no sepsis group. CRP was positive in 84.21% (16/19) of cases, while mESR was positive in 78.95% of cases of definite sepsis. [Table/Fig-4] compares the values of expression of flow cytometric parameters among the sepsis group, no sepsis group, and healthy controls, while [Table/Fig-5] illustrates the ROC Curve of nCD-64, mHLA-DR, and sepsis index in these groups.

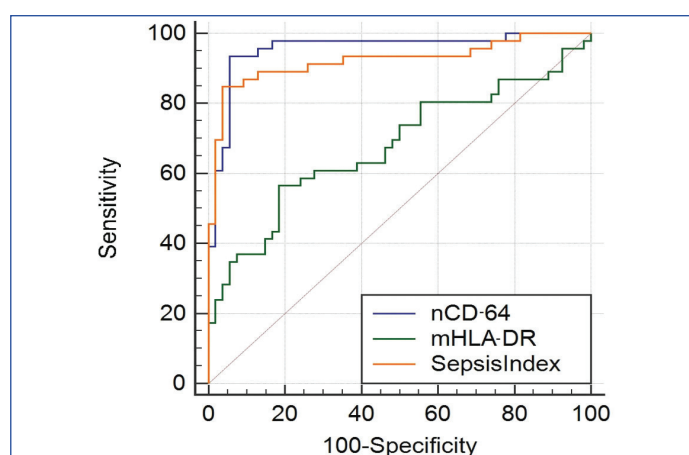
Laboratory parameter	Definite sepsis (n=19) n (%)	Probable sepsis (n=27) n (%)	No sepsis (n=24) n (%)	p-value
TLC (<5000/mm <sup>3</sup> )	7 (36.84%)	11 (40.74%)	2 (8.33%)	<b>0.025</b>
ANC (<1800/mm <sup>3</sup> )	4 (21.05%)	4 (14.81%)	0 (0%)	0.076
I:T Ratio (>0.20)	14 (73.68%)	15 (55.56%)	0 (0%)	<b>&lt;0.001</b>
Platelet (<1 lac)	9 (47.37%)	6 (22.22%)	1 (4.17%)	<b>0.004</b>
Micro ESR (raised*)	15 (78.95%)	14 (51.85%)	9 (37.50%)	<b>0.043</b>
CRP (positive)	16 (84.21%)	24 (88.89%)	8 (33.33%)	<b>&lt;0.001</b>
nCD-64 (positive)	19 (100%)	24 (88.89%)	3 (12.50%)	<b>&lt;0.001</b>
mHLA DR (positive)	12 (63.16%)	16 (59.26%)	13 (54.17%)	0.834
Sepsis Index (nCD-64/m HLA DRx100)	18 (94.74%)	23 (85.19%)	9 (37.50%)	<b>&lt;0.001</b>

**[Table/Fig-3]:** Laboratory parameters in study populations (Cases=70).

n (%): Number (proportion); p-value showed difference between sepsis and no sepsis group (Chi-square test)

Parameter	Definite sepsis	Probable sepsis	No sepsis	Healthy controls	p-value*
nCD-64 (MFI)	6229.37	3869.67	1525.38	1092.20	<b>&lt;0.001</b>
mHLA DR (MFI)	9136.84	10255.37	8617.00	14964.90	<b>0.034</b>
Sepsis index	213.57	78.29	19.71	8.82	<b>&lt;0.001</b>

**[Table/Fig-4]:** Comparison of flow cytometric parameters of sepsis group, no sepsis group and healthy controls.



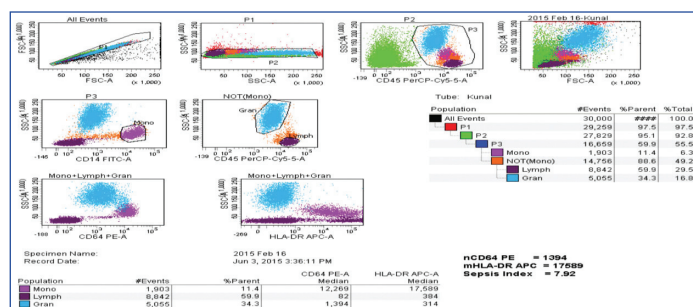
**[Table/Fig-5]:** Receiver Operating Characteristics (ROC) curve for the nCD-64, mHLA-DR and sepsis index.

On post-hoc analysis, it was observed that in the sepsis group, the expression of nCD-64 was significantly up-regulated compared to the healthy controls and the no sepsis group. However, there were no significant differences between the no sepsis group and healthy controls. While mHLA-DR did not show any significant difference between the sepsis and no sepsis groups, there was a slight down-regulation in the MFI of mHLA-DR in the sepsis group compared to the healthy controls. The sepsis index was significantly higher in the sepsis group (213.57 in definite sepsis, 78.29 in probable sepsis) compared to the no sepsis group (19.71) and the healthy controls (8.82) (p-value <0.001). The sepsis index showed a sensitivity of 94.73%, specificity of 62.50%, PPV of 66.66%, NPV of 93.75%, and accuracy of 76.74% in the definite sepsis group [Table/Fig-6].

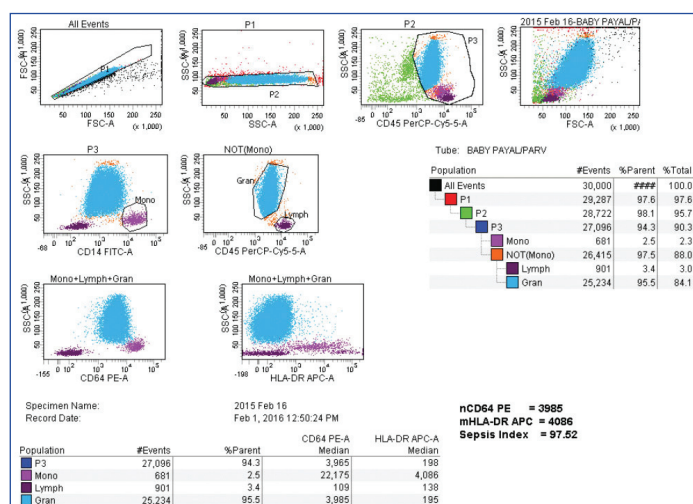
Parameter	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
nCD-64	100	88	86	100	93
mHLA-DR	63	46	48	61	53
Sepsis index	95	63	67	94	77

[Table/Fig-6]: Statistical analysis of flow cytometric parameters in definite sepsis.

Blood culture was taken as the 'gold standard' for the diagnosis of neonatal sepsis. All cases of definite sepsis (19/19) had a positive nCD-64. In the probable sepsis group, nCD-64 positivity was seen in 88.89% (24/27) of cases. However, 12.50% (3/24) of cases in the no sepsis group also had a positive nCD-64. In definite sepsis, nCD-64 revealed 100% sensitivity, 87.5% specificity, 86.36% PPV, 100% NPV, and 93.02% diagnostic accuracy [Table/Fig-6]. In the definite sepsis group, mHLA-DR was positive in 63.16% (12/19) of cases, 59.26% (16/27) in probable sepsis, and 54.17% (13/24) in the no sepsis group. Out of 19 cases of definite sepsis, 94.74% (18) cases had a positive sepsis index while one case had a sepsis index less than the cut-off value [Table/Fig-6]. [Table/Fig-7] shows flow cytometric expression of nCD-64, mHLA-DR, and sepsis Index in a healthy control, while [Table/Fig-8] shows flow cytometric expression of nCD-64, mHLA-DR, and Sepsis Index in a case of definite sepsis.



[Table/Fig-7]: Flow cytometric expression of nCD-64, mHLA-DR and sepsis index in a healthy control.



[Table/Fig-8]: Flow cytometric expression of nCD-64, mHLA-DR and sepsis index in a case of definite sepsis.

## DISCUSSION

Sepsis remains a significant cause of neonatal mortality and morbidity, especially in low and middle-income countries. Neonatal sepsis presents with non-specific signs and symptoms that necessitate tests to confirm the diagnosis. Early and accurate diagnosis of infection will improve clinical outcomes and decrease overuse of antibiotics. Current diagnostic methods rely on conventional culture methods which are time consuming and may delay critical therapeutic decisions. Non-culture-based newer techniques may overcome some of the limitations seen with culture-based techniques [16].

The present study detected that the blood culture positivity rate was 27.14% (19/70), which was similar to Fang DH et al., (26.25%) [17]. However, Jonnala RN et al., reported a higher blood culture positivity rate of 57.10% compared to the present study [18]. These variations in the results of blood culture in different studies may be attributed to differences in blood volume withdrawn, blood sampling time, blood culture techniques, severity of infection, and exposure to antibiotics. Misdiagnosis could be another factor due to some similarities between the clinical signs of sepsis and other diseases like metabolic disorders.

*Staphylococcus* was the most common organism identified in 31.58% of the total culture-positive cases, followed by Coagulase Negative *Staphylococci* (CoNS) at 26.32%. This result was in concordance with Marchant E et al., who found that gram-positive organisms accounted for the majority of neonatal sepsis cases (70%), while sepsis due to gram-negative organisms accounted for 15-20% [19]. In the present study, blood culture revealed a sensitivity of 41.30%, specificity of 100%, positive predictive value (PPV) of 100%, and negative predictive value (NPV) of 47.05% for the diagnosis of neonatal sepsis.

There was a predominance of male neonates (52.63%) in the definite sepsis group and 66.67% in the probable sepsis group, which is similar to what was observed by Aftab R and Iqbal I (63.4% male) [20]. In the present study, laboratory parameters between the sepsis group and the no sepsis group showed that the incidence of abnormal TLC, ANC, I:T Ratio, platelet count, micro ESR, and CRP was significantly higher in the definitive sepsis and all sepsis groups compared to the no sepsis group (<0.05). These results were in agreement with Bhandari V et al., and Mondal SK et al., who found that the haematological profiles of neonates with septicaemia were characterised by abnormal white cell count, a high immature to total neutrophil ratio, and a lower platelet count [21,22].

The expression of CD-64 is effective in diagnosing neonatal sepsis, but its diagnostic efficacy varied from 26-97% in sensitivity and 71-100% in specificity, possibly due to population heterogeneity, assay methodologies, and case classification criteria [23]. Median Fluorescence Intensity (MFI) was considered as the reporting parameter. Diagnostic parameters were calculated based on the mean+2SD of healthy controls for upregulated parameters and the 10<sup>th</sup> percentile in the case of downregulated parameters.

The results of this study showed a statistically significant difference between the sepsis and no sepsis groups regarding the percentage of expression of CD-64 on neutrophils. In the definite sepsis group, the MFI of nCD-64 was higher compared to the no sepsis group and healthy controls. In the present study, nCD-64 revealed 100% sensitivity, 87.5% specificity, 86.36% PPV, 100% NPV, and 93.02% diagnostic accuracy in culture-positive sepsis. These results are in concordance with Adib M et al., who found that CD-64 expression was significantly higher in the group with sepsis and revealed a sensitivity of 92.3%, specificity of 100%, PPV of 100%, and NPV of 88%, respectively [24].

In the case of mHLA-DR expression, there was no significant difference between the sepsis group and the no sepsis group. The down-regulation of mHLA-DR in the no sepsis group could

be due to other factors such as meconium aspiration syndrome and respiratory distress in neonates. However, there was a slight downregulation of mHLA-DR expression in the sepsis group compared to the healthy neonates. However, the downregulation of mHLA-DR observed in the present study alone showed poor diagnostic utility. In the definite sepsis group, mHLA DR revealed a sensitivity of 63.15%, specificity of 45.83%, PPV of 48%, NPV of 61.11%, and accuracy of 53.48%. The downregulation of mHLA-DR in definite sepsis was more pronounced compared to healthy controls. This was significantly lower in the severely septic neonates who subsequently succumbed to the illness. Similar results were observed by Sedlackova L et al., and Juskewitch JE et al., in their study and concluded that downregulation of HLA-DR expression on monocytes can be a useful indicator in septic patients when considered along with other markers [25,26].

The sepsis index showed a significant difference between various groups, including definite sepsis, probable sepsis, no sepsis group, and healthy controls. The sepsis index was calculated in all the neonates in this study and found a sensitivity of 94.73%, specificity of 62.50%, PPV of 66.66%, NPV of 93.75%, and accuracy of 76.74% in the definite sepsis group. Therefore, the sepsis index can be a useful marker of neonatal sepsis, as also suggested by Pardhan R et al., who found a sensitivity and specificity of 73.01% and 72.22%, respectively [15].

### Limitation(s)

The present study suggested that nCD-64 expression is a very sensitive and moderately specific marker for early and late-onset neonatal sepsis and can be used independently as a diagnostic marker for neonatal sepsis. However, a limitation of the study was that it is very expensive and is available only in a few tertiary care centers. mHLA-DR expression was definitely low in the sepsis group, but prognostic utility is not established in the present study as follow-up samples were not included.

### CONCLUSION(S)

Flow cytometric assessment of neutrophil CD-64 may be considered a rapid and reliable marker for the diagnosis of bacterial neonatal sepsis. It is also useful to identify a separate group among culture-negative sick neonates and may be useful to guide early antibiotic administration, especially in these neonates. mHLA-DR may be beneficial for monitoring patients at a later point in time for the identification of delayed immunosuppression in neonatal sepsis.

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