RESEARCH PAPER

EFFICACY OF NEUTROPHIL CD64 IN DISTINGUISHING BACTERIAL INFECTION FROM INFLAMMATION IN SEVERE ALCOHOLIC HEPATITIS SATISFYING SYSTEMIC INFLAMMATORY RESPONSE SYNDROME (SIRS) CRITERIA :A PROSPECTIVE OBSERVATIONAL STUDY

Dr. Nitesh Balaji Nalabale[1], Dr Santosh Dhananjay Hajare[2], Dr Panchlingappa Betageri[3], Dr. Tharun Shiva Pasula[4]

[1]Senior Resident, Department of Medical Gastroenterology, JNMC, Belagavi, Jawaharlal lal Medical College, Belagavi, Karnataka, 590010, India.

[2]Hod Of Medical Gastroenterology, Department of Medical Gastroenterology, Jawaharlal Neharu Medical College, Nehru Nagar Belagavi, 590010, India.

[3]Assistant Professor, Department of Medical Gastroenterology, JNMC, Belagavi, India.

[4]Senior Resident, Department of Gastroenterology, JNMC Belagavi, India.

Corresponding author addresses: E-mail id/ Social Media Link

Dr. Nitesh Balaji Nalabale, Senior Resident, Department of Medical Gastroenterology, JNMC, Belagavi, Jawaharlal lal Medical College, Belagavi, Karnataka, 590010, India.

Email: niteshnalabale26@gmail.com

ABSTRACT

Background: Severe alcoholic hepatitis is frequently accompanied by systemic inflammatory response syndrome, even without infection. Because bacterial infection and sterile hepatic inflammation may have similar clinical and laboratory presentations, early differentiation is difficult. Neutrophil CD64 is rapidly upregulated during bacterial infection and may offer greater specificity than conventional inflammatory biomarkers. **Aim:** To evaluate the efficacy of quantitative neutrophil CD64 expression in distinguishing bacterial infection from sterile inflammation in patients with severe alcoholic hepatitis satisfying SIRS criteria. **Materials and Methods:** This prospective observational study included 67 patients with severe alcoholic hepatitis satisfying SIRS criteria. Based on clinical, microbiological, biochemical and radiological evaluation, 34 patients were classified as having bacterial infection and 33 as having sterile inflammation. Neutrophil CD64 expression was measured by flow cytometry and expressed as the percentage of neutrophils expressing CD64. Serum procalcitonin, high-sensitivity C-reactive protein, erythrocyte sedimentation rate, total leukocyte count and neutrophil-to-lymphocyte ratio were also evaluated. Receiver-operating-characteristic analysis was performed to determine optimal cut-offs and diagnostic accuracy. A p value <0.05 was considered statistically significant. **Results:** Mean nCD64 expression was significantly higher in the bacterial-infection group than in the sterile-inflammation group (46.27±33.12% versus 11.75±16.37%). The mean difference was 34.52 percentage points (95% CI: 21.75–47.29; $p<0.001$). At the optimal cut-off of ≥27%, nCD64 demonstrated 76.5% sensitivity, 90.9% specificity, 89.7% positive predictive value, 78.9% negative predictive value and 83.6% overall accuracy. Its AUC was 0.816 (95% CI: 0.709–0.923; $p<0.001$), and the diagnostic odds ratio was 32.50 (95% CI: 7.80–135.41). The NLR had an AUC of 0.804, followed by total leukocyte count at 0.781, serum procalcitonin at 0.719, hs-CRP at 0.577 and ESR at 0.463. Mean and median CD64 fluorescence-intensity measurements did not differ significantly between the groups. **Conclusion:** The percentage of neutrophils expressing CD64 was a useful biomarker for distinguishing bacterial infection from sterile inflammation in patients with severe alcoholic hepatitis satisfying SIRS criteria. An nCD64 threshold of ≥27% provided high specificity and good overall diagnostic accuracy. Neutrophil CD64 may be incorporated as an adjunct to microbiological, radiological and clinical assessment, but larger multicentre studies are required before its routine independent use.

KEYWORDS: Neutrophil CD64; severe alcoholic hepatitis; bacterial infection.

How to cite this article: Nitesh Balaji Nalabale1 Dr Santosh Dhananjay Hajare2 Dr Panchlingappa Betageri3 Dr. Tharun Shiva Pasula4. EFFICACY OF NEUTROPHIL CD64 IN DISTINGUISHING BACTERIAL INFECTION FROM INFLAMMATION IN SEVERE ALCOHOLIC HEPATITIS SATISFYING SYSTEMIC INFLAMMATORY RESPONSE SYNDROME (SIRS) CRITERIA :A PROSPECTIVE OBSERVATIONAL STUDY. Int J Drug Deliv Technol. 2026;16(80s):420-431. DOI: 10.25258/ijddt.16.80s.50.

INTRODUCTION

Alcohol-associated hepatitis is an acute inflammatory manifestation of alcohol-associated liver disease, characterized by recent onset or worsening of jaundice following prolonged heavy alcohol consumption. Severe alcohol-associated hepatitis, commonly defined by a Maddrey Discriminant Function (mDF)

score ≥32 or a Model for End-Stage Liver Disease (MELD) score >20, is associated with substantial short-term mortality [1,2]. Systemic inflammation is a prominent feature of severe alcoholic hepatitis, and many patients satisfy systemic inflammatory response syndrome (SIRS) criteria even in the absence of infection. At the same time, impaired intestinal barrier function, bacterial translocation, cirrhosis-associated immune dysfunction, malnutrition and frequent hospitalization make these patients highly susceptible to bacterial infections [2,3]. Differentiating sterile inflammation from bacterial infection is therefore clinically important because both conditions may present with fever, tachycardia, tachypnoea and leukocytosis. Moreover, corticosteroid therapy may benefit selected patients with severe alcoholic hepatitis, but its initiation requires careful evaluation for active infection [2]. Conventional investigations such as total leukocyte count, neutrophil-to-lymphocyte ratio, erythrocyte sedimentation rate, C-reactive protein and serum procalcitonin have limited discriminatory ability in this population because their concentrations may be altered by severe hepatic inflammation, hepatocellular injury and immune dysfunction. Microbiological culture remains important for confirming infection; however, culture results are delayed, sensitivity may be reduced following prior antibiotic administration and clinically important infections may remain culture-negative. Neutrophil CD64, a high-affinity Fc-gamma receptor I, is normally expressed at low levels on circulating neutrophils. Following bacterial infection, inflammatory mediators particularly interferon-gamma and granulocyte colony-stimulating factor rapidly upregulate its expression, which can be quantified by flow cytometry [4]. A previous study involving patients with severe alcoholic hepatitis fulfilling SIRS criteria reported that the percentage of neutrophils expressing CD64 was significantly higher among patients with bacterial infection. At an nCD64 cut-off of 27%, the test demonstrated 94% sensitivity, 81% specificity and an area under the receiver-operating-characteristic curve of 0.95, outperforming procalcitonin, C-reactive protein, total leukocyte count and neutrophil-to-lymphocyte ratio [5].

AIM

To evaluate the efficacy of neutrophil CD64 expression in distinguishing bacterial infection from sterile inflammation in patients with severe alcoholic hepatitis satisfying SIRS criteria.

OBJECTIVES

1. To compare quantitative neutrophil CD64 expression between patients with severe alcoholic hepatitis and SIRS with and without bacterial infection.
2. To determine the diagnostic accuracy and optimal cut-off value of neutrophil CD64 for identifying bacterial infection.
3. To compare the diagnostic performance of neutrophil CD64 with serum procalcitonin, hs-CRP, ESR, total leukocyte count and neutrophil-to-lymphocyte ratio.

MATERIALS AND METHODOLOGY

Source of Data

The study population comprised patients admitted to or attending the Department of Medical Gastroenterology, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belagavi, who were diagnosed with severe alcoholic hepatitis and satisfied SIRS criteria. Eligible participants were recruited consecutively after obtaining written informed consent.

Study Design

The study was conducted as a hospital-based, prospective observational analytical study. After completing the infection work-up, participants were classified as having severe alcoholic hepatitis with bacterial infection or severe alcoholic hepatitis with sterile inflammation but without demonstrable infection.

Study Location

The study was conducted in the Department of Medical Gastroenterology in collaboration with the Departments of Microbiology, Pathology/Biochemistry and the Flow Cytometry Laboratory at KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belagavi, Karnataka.

Study Duration

The study was conducted over 18 months, from 1 September 2024 to 1 March 2026.

Sample Size

A total of 67 eligible patients with severe alcoholic hepatitis satisfying SIRS criteria were included. Participants were recruited through consecutive sampling and were subsequently classified into infection and non-infection groups according to prespecified clinical, microbiological and radiological criteria. Because the requested sample size was 30, the study was considered an exploratory diagnostic-accuracy study, and estimates were reported with 95% confidence intervals.

Inclusion Criteria

Patients were included if they:

1. Were aged 18–75 years, irrespective of sex.
2. Provided written informed consent personally or through a legally authorized representative.
3. Fulfilled the NIAAA clinical criteria for alcoholic hepatitis, including recent onset or worsening of jaundice, a history of prolonged heavy alcohol consumption, serum bilirubin

>3 mg/dL, aspartate aminotransferase >50 U/L but <400 U/L and an AST:ALT ratio >1.5.

4. Had severe alcoholic hepatitis defined by an mDF score ≥32 and/or MELD score >20.
5. Satisfied at least two SIRS criteria:
    - Temperature >38°C or <36°C;
    - Heart rate >90 beats/minute;
    - Respiratory rate >20 breaths/minute; or
    - Total leukocyte count >12,000/μL, <4,000/μL or >10% immature forms.

Exclusion Criteria

Patients were excluded if they:

1. Had an absolute neutrophil count <1,500 cells/μL.
2. Had received granulocyte colony-stimulating factor before enrolment.
3. Had received complementary or alternative medicines likely to affect inflammatory or haematological parameters.
4. Had suspected or confirmed mycobacterial or invasive fungal infection.
5. Were receiving immunosuppressive treatment other than corticosteroids or were receiving chemotherapy.
6. Had hepatocellular carcinoma or another active malignancy.
7. Had undergone major surgery or sustained significant trauma within three months before enrolment.
8. Had another acute systemic inflammatory disease unrelated to liver disease that could independently alter neutrophil CD64 expression.
9. Declined consent or had an inadequate blood specimen for flow-cytometric analysis.

Data Collection

Data were collected prospectively using a predesigned and pretested study proforma. The information recorded included age, sex, occupation, alcohol type, average daily alcohol intake, duration of alcohol use, date of last alcohol consumption, presenting symptoms, comorbidities, previous antibiotic exposure and relevant treatment history. Clinical examination included temperature, pulse rate, respiratory rate, blood pressure, oxygen saturation, nutritional status, pallor, icterus, oedema, ascites, hepatic encephalopathy and evidence of localized infection.

Laboratory data included complete blood count, differential leukocyte count, neutrophil-to-lymphocyte ratio, peripheral smear, ESR, hs-CRP, serum procalcitonin, liver function tests, renal function tests, electrolytes, serum albumin, prothrombin time/INR and viral markers. The mDF and MELD scores were calculated from admission parameters. Findings from blood, urine, sputum, ascitic fluid, pus and other clinically indicated cultures were recorded. Chest radiography, abdominal ultrasonography and other imaging findings were also documented.

Each participant was assigned a unique study identification number. Personal identifiers were kept separately from the analytical dataset, and only coded information was used for analysis.

Procedure and Methodology

After admission, potentially eligible patients were screened for alcoholic hepatitis using the NIAAA clinical criteria. Other causes of acute liver injury, including viral hepatitis, drug-induced liver injury, biliary obstruction, autoimmune liver disease and ischemic hepatitis, were excluded through history, biochemical investigations, viral markers and imaging as clinically indicated. Severity was assessed using the mDF and MELD scores.

Participants were evaluated for SIRS using temperature, heart rate, respiratory rate and leukocyte count recorded at enrolment. Patients satisfying at least two SIRS criteria underwent a structured infection work-up before or as close as possible to the administration of antibiotics.

Bacterial infection was categorized as follows:

- **Proven infection:** a pathogenic organism was isolated from blood, urine, ascitic fluid, sputum, pus or another appropriate clinical specimen.
- **Probable infection:** clinical and/or radiological evidence of infection was present despite negative cultures, particularly when antibiotics had been administered before referral.
- **No infection/sterile inflammation:** clinical evaluation, chest imaging, urinalysis, ascitic fluid examination when indicated and relevant cultures did not reveal an infectious focus.

Spontaneous bacterial peritonitis was diagnosed when the ascitic fluid polymorphonuclear leukocyte count was ≥250 cells/μL, irrespective of culture positivity, in the absence of a surgically treatable intra-abdominal source. Lower respiratory tract infection was diagnosed from compatible respiratory symptoms and signs together with a new infiltrate, consolidation or lung abscess on chest radiography or computed tomography. Urinary tract infection was diagnosed using urinary symptoms, pyuria and/or significant bacterial growth in urine culture. Skin and soft-tissue infection was diagnosed from cellulitis, an abscess or purulent discharge supported by microbiological evaluation whenever obtainable.

Participants with proven or probable bacterial infection were included in the infection group, while those with a negative infection work-up were included in the non-infection or sterile-inflammation group. Clinicians responsible for patient management were not required to delay necessary antibiotics while awaiting culture or CD64 results. Laboratory personnel performing the neutrophil CD64 assay were blinded to the clinical infection classification.

Sample Processing

Approximately 6 mL of peripheral venous blood was collected under aseptic precautions at enrolment, preferably before the first dose of antibiotics. Three millilitres were placed in an EDTA tube for complete blood count, peripheral smear and neutrophil CD64 measurement, while the remaining 3 mL were collected in a plain or serum-separator tube for hs-CRP and serum procalcitonin estimation.

The complete blood count and differential leukocyte count were measured using the Sysmex XN-1000 automated haematology analyser. Peripheral blood smears were examined microscopically when clinically indicated. The neutrophil-to-lymphocyte ratio was calculated by dividing the absolute neutrophil count by the absolute lymphocyte count. ESR was determined using the Westergren method.

Hs-CRP was measured using an immunoturbidimetric assay on the Roche Cobas platform. Serum procalcitonin was measured by electrochemiluminescence immunoassay, with 0.5 ng/mL used as a conventional indicator of possible bacterial infection, while the continuous procalcitonin value was retained for ROC analysis.

Neutrophil CD64 was quantified using a Beckman Coulter DxFLEX flow cytometer. Approximately 100 µL of well-mixed EDTA-anticoagulated whole blood was incubated with fluorochrome-conjugated anti-human CD64 and anti-CD45 monoclonal antibodies for approximately 20 minutes at room temperature in the dark. Red blood cells were lysed by adding approximately 0.5 mL of OptiLyse reagent, followed by vortex mixing and further incubation for 20 minutes. The cells were washed twice with phosphate-buffered saline and resuspended for acquisition.

Leukocytes were identified using CD45 expression and side-scatter characteristics, and neutrophils were appropriately gated. CD64 expression was recorded as:

- Percentage of neutrophils expressing CD64 (nCD64%);
- Mean fluorescence intensity; and
- Median fluorescence intensity.

Appropriate instrument calibration, compensation and internal quality-control procedures were performed according to the laboratory's standard operating procedures. Samples were processed as early as possible after collection to minimize changes associated with storage.

Statistical Methods

Data were entered into a spreadsheet, checked for completeness and consistency, and analysed using an appropriate statistical software package. Categorical variables were summarized as frequencies and percentages, while continuous variables were summarized as mean with standard deviation when normally distributed and median with interquartile range when non-normally distributed. Normality was assessed using the Shapiro–Wilk test and graphical methods.

Continuous variables were compared between infection and non-infection groups using the independent-samples Student's *t* test or Mann–Whitney *U* test, as appropriate. Categorical variables were compared using the chi-square test or Fisher's exact test. Correlations between nCD64 and other biomarkers were assessed using Pearson's or Spearman's correlation coefficient.

Receiver-operating-characteristic curves were constructed for nCD64%, CD64 fluorescence intensity, serum procalcitonin, hs-CRP, ESR, total leukocyte count and neutrophil-to-lymphocyte ratio. Diagnostic performance was expressed as area under the ROC curve with 95% confidence intervals. The optimal nCD64 cut-off was identified using the Youden index. Sensitivity, specificity, positive predictive value, negative predictive value, positive and negative likelihood ratios and overall diagnostic accuracy were calculated with 95% confidence intervals. All statistical tests were two-tailed, and $p<0.05$ was considered statistically significant.

OBSERVATION AND RESULTS

Table 1: Overall efficacy of neutrophil CD64 for distinguishing bacterial infection from sterile inflammation in severe alcoholic hepatitis with SIRS (N=67)

Infection status	nCD64 positive ≥27%, n (%)	nCD64 negative <27%, n (%)	Total, n (%)	Association estimate (95% CI)	Test of significance	P value
Bacterial infection	26 (76.5%)	8 (23.5%)	34 (50.7%)	OR= 32.50 (7.80 – 135.41)	χ^2=30.97	<0.001

Sterile inflammation	3 (9.1%)	30 (90.9%)	33 (49.3%)	Reference		
Total	**29 (43.3%)**	**38 (56.7%)**	**67 (100.0%)**			

Table 1 demonstrates a strong association between increased neutrophil CD64 expression and bacterial infection among patients with severe alcoholic hepatitis satisfying SIRS criteria. Of the 34 patients with bacterial infection, 26 (76.5%) had nCD64 expression ≥27%, compared with only 3 (9.1%) of the 33 patients with sterile inflammation. Conversely, nCD64 expression <27% was observed in 8 (23.5%) patients with bacterial infection and 30 (90.9%) patients with sterile inflammation. Overall, 29 (43.3%) patients were nCD64-positive and 38 (56.7%) were nCD64-negative. Patients with nCD64 ≥27% had 32.50 times higher odds of bacterial infection than those with nCD64 <27% (OR=32.50; 95% CI: 7.80–135.41). The association was highly statistically significant (χ^2=30.97, p<0.001), indicating that an nCD64 threshold of ≥27% was effective in distinguishing bacterial infection from sterile inflammation.

Table 2: Comparison of quantitative neutrophil CD64 expression between bacterial infection and sterile-inflammation groups (N=67)

Quantitative CD64 parameter	**Infection group (n=34), Mean (SD)**	**Sterile-inflammation group (n=33), Mean (SD)**	**Mean difference (95% CI)**	**Test of significance**	**P value**
nCD64-expressing neutrophils, %	46.27 (33.12)	11.75 (16.37)	34.52 (21.75–47.29)	Welch's t=5.43	<0.001
CD64 mean fluorescence intensity	3895 (1145)	3843 (1235)	52 (−530 to 634)	Welch's t=0.18	0.859
CD64 median fluorescence-intensity measure	3244 (882)	3022 (611)	222 (−148 to 592)	Welch's t=1.20	0.235

Table 2 compares quantitative CD64 measurements between patients with bacterial infection and those with sterile inflammation. The mean percentage of nCD64-expressing neutrophils was substantially higher in the infection group than in the sterile-inflammation group (46.27±33.12% versus 11.75±16.37%). The mean difference was 34.52 percentage points (95% CI: 21.75–47.29), which was highly statistically significant (Welch's t=5.43, p<0.001). However, CD64 mean fluorescence intensity was similar between the infection and sterile-inflammation groups (3895±1145 versus 3843±1235), with a mean difference of only 52 units (95% CI: −530 to 634; Welch's t=0.18, p=0.859). Similarly, the CD64 median fluorescence-intensity measure was numerically higher in the infection group (3244±882) than in the sterile-inflammation group (3022±611), but the mean difference of 222 units was not statistically significant (95% CI: −148 to 592; Welch's t=1.20, p=0.235).

Table 3: Diagnostic accuracy and optimal cut-off value of nCD64 for identifying bacterial infection (N=67)

Diagnostic parameter	**Result**	**95% CI**	**Test statistic**	**P value**
Optimal nCD64 cut-off	≥27%		Youden index=0.674	
Sensitivity	76.5% (26/34)	60.0% – 87.6%		
Specificity	90.9% (30/33)	76.4% – 96.9%		
Positive predictive value	89.7% (26/29)	73.6% – 96.4%		
Negative predictive value	78.9% (30/38)	63.7% – 88.9%		
Overall diagnostic accuracy	83.6% (56/67)	72.9% – 90.6%		
Area under ROC curve	0.816	0.709 – 0.923	ROC z=5.79†	<0.001

Diagnostic odds ratio	32.50	7.80–135.41	χ^2=30.97	<0.001

†The ROC *z* statistic was calculated against the null hypothesis AUC=0.50.

Cut-off-wise diagnostic performance of nCD64

nCD64 cut-off	Sensitivity	Specificity	PPV	NPV	Youden index
≥15%	91.2%	57.6%	68.9%	86.4%	0.488
≥20%	85.3%	72.7%	76.3%	82.8%	0.580
≥27%	**76.5%**	**90.9%**	**89.7%**	**78.9%**	**0.674**

Table 3 presents the diagnostic accuracy of nCD64 for identifying bacterial infection. At the optimal cut-off value of ≥27%, nCD64 demonstrated a sensitivity of 76.5% (95% CI: 60.0%–87.6%), indicating that approximately three-fourths of patients with bacterial infection were correctly identified. Its specificity was 90.9% (95% CI: 76.4%–96.9%), showing that it correctly excluded bacterial infection in most patients with sterile inflammation. The positive predictive value was 89.7% (95% CI: 73.6%–96.4%), while the negative predictive value was 78.9% (95% CI: 63.7%–88.9%). The overall diagnostic accuracy was 83.6% (95% CI: 72.9%–90.6%). The area under the ROC curve was 0.816 (95% CI: 0.709–0.923), which was significantly greater than the non-discriminatory value of 0.50 (ROC z=5.79, p<0.001). The diagnostic odds ratio was 32.50 (95% CI: 7.80–135.41; χ^2=30.97, p<0.001), further supporting the strong diagnostic association between increased nCD64 and bacterial infection.

The cut-off-wise analysis showed that lowering the nCD64 threshold increased sensitivity but reduced specificity. At ≥15%, sensitivity was highest at 91.2%, but specificity was only 57.6%, with a Youden index of 0.488. At ≥20%, sensitivity was 85.3% and specificity was 72.7%, producing a Youden index of 0.580. The ≥27% cut-off provided the highest Youden index of 0.674, with 76.5% sensitivity, 90.9% specificity, 89.7% positive predictive value and 78.9% negative predictive value. Therefore, ≥27% represented the optimal threshold because it achieved the best balance between detecting true infections and minimizing false-positive diagnoses.

Table 4: Comparison of nCD64 with conventional biomarkers for detecting bacterial infection in severe alcoholic hepatitis with SIRS (N=67)

Biomarker	Infection group (n=34), Mean (SD)	Sterile-inflammation group (n=33), Mean (SD)	Mean difference (95% CI)	Test statistic	P value
nCD64, %	46.27 (33.12)	11.75 (16.37)	34.52 (21.75–47.29)	Welch's t=5.43	<0.001
Serum procalcitonin, ng/mL	2.50 (8.89)	0.53 (0.47)	1.97 (−1.14 to 5.08)	Welch's t=1.29	0.206†
hs-CRP, mg/L	62.06 (36.06)	55.44 (49.98)	6.62 (−14.75 to 27.99)	Welch's t=0.62	0.538
ESR, mm/hour	60.47 (25.20)	65.39 (31.14)	−4.92 (−18.78 to 8.94)	Welch's t=−0.71	0.481
Total leukocyte count, cells/mm³	15,620 (5,840)	10,980 (4,120)	4,640 (2,175–7,105)	Welch's t=3.77	<0.001
Neutrophil-to-lymphocyte ratio	9.15 (7.10)	5.28 (4.20)	3.87 (1.02–6.72)	Welch's t=2.72	0.009

†Procalcitonin was markedly right-skewed. The attached document reported p=0.036.

ROC comparison of diagnostic performance

Biomarker	Optimal cut-off	AUC (95% CI)	Sensitivity	Specificity	Accuracy	ROC test P value
nCD64	**≥27%**	**0.816 (0.709–0.923)**	**76.5%**	**90.9%**	**83.6%**	**<0.001**
NLR	≥5.8	0.804 (0.	79.4%	81.8%	80.6%	<0.001

		693–0.915)				
TLC	≥13,200/mm³	0.781 (0.667–0.895)	73.5%	75.8%	74.6%	<0.001
Serum procalcitonin	≥0.50 ng/mL	0.719 (0.595–0.844)	82.4%	60.6%	71.6%	0.003
hs-CRP	≥48.1 mg/L	0.577 (0.438–0.716)	67.6%	54.5%	61.2%	0.289
ESR	≥56 mm/hour	0.463 (0.322–0.603)	64.7%	42.4%	53.7%	0.612

Table 4 compares nCD64 with conventional inflammatory biomarkers. The mean nCD64 percentage was significantly higher in patients with bacterial infection than in those with sterile inflammation (46.27±33.12% versus 11.75±16.37%). The corresponding mean difference was 34.52 percentage points (95% CI: 21.75–47.29; Welch's t=5.43, p<0.001). Mean serum procalcitonin was also numerically higher in the infection group (2.50±8.89 ng/mL) than in the sterile-inflammation group (0.53±0.47 ng/mL). However, the Welch's t test did not show a statistically significant difference (mean difference=1.97 ng/mL; 95% CI: −1.14 to 5.08; t=1.29, p=0.206), probably because of its markedly skewed distribution and large variability in the infection group. Therefore, median and interquartile range with a Mann–Whitney U test would be more appropriate for procalcitonin.

Hs-CRP was slightly higher in patients with bacterial infection than in those with sterile inflammation (62.06±36.06 versus 55.44±49.98 mg/L), but the difference was not statistically significant (mean difference=6.62 mg/L; 95% CI: −14.75 to 27.99; Welch's t=0.62, p=0.538). ESR was marginally lower in the infection group (60.47±25.20 mm/hour) than in the sterile-inflammation group (65.39±31.14 mm/hour), with no significant difference (mean difference=−4.92 mm/hour; 95% CI: −18.78 to 8.94; Welch's t=−0.71, p=0.481). In contrast, total leukocyte count was significantly higher in the infection group (15,620±5,840 cells/mm³) than in the sterile-inflammation group (10,980±4,120 cells/mm³). The mean difference was 4,640 cells/mm³ (95% CI: 2,175–7,105; Welch's t=3.77, p<0.001). The neutrophil-to-lymphocyte ratio was also significantly elevated in the infection group (9.15±7.10 versus 5.28±4.20), with a mean difference of 3.87 (95% CI: 1.02–6.72; Welch's t=2.72, p=0.009).

ROC analysis showed that nCD64 had the highest discriminatory performance, with an AUC of 0.816 (95% CI: 0.709–0.923; p<0.001). At a cut-off of ≥27%, it provided 76.5% sensitivity, 90.9% specificity and 83.6% overall accuracy. NLR was the second-best biomarker, with an AUC of 0.804 (95% CI: 0.693–0.915; p<0.001); at a cut-off of ≥5.8, its sensitivity, specificity and accuracy were 79.4%, 81.8% and 80.6%, respectively. TLC showed good discriminatory ability, with an AUC of 0.781 (95% CI: 0.667–0.895; p<0.001). A TLC threshold of ≥13,200 cells/mm³ provided 73.5% sensitivity, 75.8% specificity and 74.6% accuracy.

Serum procalcitonin had an AUC of 0.719 (95% CI: 0.595–0.844; p=0.003). Although its sensitivity at ≥0.50 ng/mL was relatively high at 82.4%, its specificity was only 60.6%, resulting in an accuracy of 71.6%. Hs-CRP demonstrated poor diagnostic performance, with an AUC of 0.577 (95% CI: 0.438–0.716; p=0.289), while ESR had no meaningful discriminatory ability, with an AUC of 0.463 (95% CI: 0.322–0.603; p=0.612).

DISCUSSION

Table 1: Overall efficacy of neutrophil CD64

The present study demonstrated a strong association between neutrophil CD64 positivity and bacterial infection in patients with severe alcoholic hepatitis satisfying SIRS criteria. At an nCD64 threshold of ≥27%, 76.5% of patients with bacterial infection were positive, compared with only 9.1% of patients with sterile inflammation. Patients with nCD64 ≥27% had 32.50-fold higher odds of bacterial infection (95% CI: 7.80–135.41; χ^2=30.97, p<0.001). These findings support nCD64 as a comparatively infection-specific marker in a clinical setting where fever, leukocytosis and SIRS may result from either bacterial infection or alcohol-induced sterile hepatic inflammation.

Pandey et al. (2021)[1], in the most directly comparable study, evaluated 58 patients with severe alcoholic hepatitis and infection, 70 without infection and 20 healthy controls. Median nCD64 expression was 76.2% in infected patients, compared with 16.0% in

non-infected patients and 7.05% in healthy controls. At the same ≥27% cut-off, they reported 94% sensitivity, 81% specificity and an AUC of 0.95. The present study reproduced the same optimal cut-off and similarly demonstrated a marked separation between infection and sterile inflammation, although sensitivity was lower and specificity was higher than in Pandey et al. Differences in disease severity, infection definitions, previous antibiotic exposure, timing of sampling and flow-cytometry procedures may explain this variation.

The high prevalence of bacterial infection in the present study 34 of 67 patients (50.7%) was consistent with Karakike et al. (2017)[2], who reported that infectious complications occur in approximately 50% of patients with severe alcoholic hepatitis. Michelena et al. (2015)[3] demonstrated that systemic inflammation is common in alcoholic hepatitis and is independently associated with multiorgan failure and mortality. Therefore, SIRS alone cannot reliably identify bacterial infection. Maiwall et al. (2016)[4] similarly found that SIRS at admission predicted acute kidney injury and 90-day mortality in severe alcoholic hepatitis, reinforcing the importance of distinguishing infection-related SIRS from severe sterile inflammation.

The clinical importance of early infection recognition is further supported by the STOPAH trial reported by Thursz et al. (2015)[5], in which infection was an important complication and prednisolone increased the risk of serious infection. Vergis et al. (2017)[6] subsequently reported that prednisolone treatment was associated with increased susceptibility to serious and post-treatment infections. Current clinical guidance therefore recommends comprehensive infection screening before corticosteroid initiation in severe alcohol-associated hepatitis [7]. The strong association observed in the present study suggests that nCD64 could complement cultures, imaging and clinical assessment when deciding whether bacterial infection is sufficiently likely to justify antimicrobial therapy or postpone corticosteroid treatment.

Table 2: Quantitative nCD64 expression

The mean percentage of nCD64-expressing neutrophils was significantly higher in the bacterial-infection group than in the sterile-inflammation group (46.27±33.12% versus 11.75±16.37%). The mean difference of 34.52 percentage points was both clinically and statistically significant (95% CI: 21.75–47.29; Welch's t=5.43, p<0.001). This finding indicates that the proportion of activated neutrophils expressing CD64 may be more informative than the absolute fluorescence intensity of CD64 expression.

Pandey et al. (2021)[1] also reported substantially higher nCD64 percentages among infected patients with severe alcoholic hepatitis. Their median value of 76.2% in the infection group was higher than the mean of 46.27% in the present study, but the non-infection values were comparable 16.0% in their study and 11.75% in the present study. These findings consistently show that resting neutrophils in sterile inflammatory states express relatively little CD64, whereas bacterial cytokine signalling rapidly induces CD64 expression.

Wang et al. (2015)[8], in a meta-analysis of adult patients with sepsis, reported pooled nCD64 sensitivity of 88%, specificity of 90%, a positive likelihood ratio of 11.56 and a high summary AUC. Cong et al. (2021)[9] similarly reported pooled sensitivity and specificity of 88% each for nCD64 in adult sepsis. These estimates support the biological and diagnostic relevance of increased CD64 expression, although most contributing studies involved general critical-care populations rather than patients with severe alcoholic hepatitis.

In the present study, CD64 mean fluorescence intensity did not differ significantly between the infection and sterile-inflammation groups (3895±1145 versus 3843±1235; mean difference=52; 95% CI: −530 to 634; p=0.859). The median fluorescence-intensity measure was also not significantly different (3244±882 versus 3022±611; mean difference=222; 95% CI: −148 to 592; p=0.235). In contrast, Pandey et al. (2021)[1] found significantly higher CD64 mean fluorescence intensity in infected patients than in non-infected patients 1431 versus 853, respectively. This discrepancy could reflect instrument-specific fluorescence scales, antibody clones, staining protocols, gating strategies, calibration procedures or sample-processing delays.

Patnaik et al. (2020)[10] emphasized that lack of standardization is an important limitation of nCD64 measurement, as results may be expressed as percentage positivity, mean fluorescence intensity, median fluorescence intensity or a standardized CD64 index. Pham et al. (2023)[11] also noted substantial differences in reported CD64 units and thresholds across studies. Therefore, the significant nCD64 percentage but non-significant fluorescence-intensity findings in the present study suggest that percentage positivity may be a more reproducible measure for this laboratory and patient population. Nevertheless, multicentre calibration and external validation would be needed before the ≥27% threshold could be universally adopted.

Table 3: Diagnostic accuracy and optimal cut-off

At the optimal nCD64 cut-off of ≥27%, sensitivity was 76.5%, specificity was 90.9%, positive predictive value was 89.7%, negative predictive value was 78.9% and overall accuracy was 83.6%. The AUC of 0.816 (95% CI: 0.709–0.923; p<0.001) indicated good diagnostic discrimination. The diagnostic odds ratio of

32.50 further demonstrated that a positive test was strongly associated with bacterial infection.

The specificity of 90.9% was almost identical to the 91% pooled specificity reported by Yeh et al. (2019)[12] in their meta-analysis of nCD64 for infection in adult septic syndromes. That analysis reported pooled sensitivity and specificity of approximately 87% and 89%–91%, respectively, and found nCD64 to have better overall discriminatory performance than CRP and performance comparable to or better than procalcitonin. Patnaik et al. (2020)[10] reported pooled sensitivity of 76% and specificity of 85% in their review, closely corresponding to the present sensitivity of 76.5%. Cong et al. (2021)[9] found higher pooled sensitivity and a summary AUC of approximately 0.94, suggesting that nCD64 generally has good diagnostic performance, but its accuracy varies according to patient population and diagnostic reference standard.

Cut-off analysis demonstrated the expected trade-off between sensitivity and specificity. At ≥15%, sensitivity increased to 91.2%, but specificity declined to 57.6%. Such a threshold may be suitable for initial screening when failure to identify infection is considered particularly harmful. At ≥20%, sensitivity was 85.3% and specificity was 72.7%. At ≥27%, sensitivity decreased to 76.5%, but specificity increased to 90.9%, producing the highest Youden index of 0.674. Therefore, ≥27% offered the most balanced classification in the present population.

The identical ≥27% optimal threshold reported by Pandey et al. (2021)[1] provides external support for this cut-off in severe alcoholic hepatitis. Nevertheless, Pandey et al. obtained higher sensitivity of 94% and lower specificity of 81%. The lower sensitivity in the present study may have resulted from culture-negative infection, prior antibiotics, early sampling before maximal CD64 upregulation or immunological dysfunction associated with advanced liver disease. Conversely, the higher specificity suggests that the threshold was particularly effective in excluding false-positive results arising from sterile hepatic inflammation.

Shi et al. (2016)[13], in a neonatal-sepsis meta-analysis, reported lower pooled sensitivity and specificity of 77% and 74%, respectively, illustrating that nCD64 performance is not uniform across populations. Thus, nCD64 should not be interpreted independently of age, underlying disease, assay platform and infection definition. In severe alcoholic hepatitis, the test should be used as part of a combined diagnostic assessment rather than as a replacement for cultures, ascitic-fluid examination, radiological investigations and clinical evaluation.

Table 4: Comparison with conventional biomarkers

Among the evaluated biomarkers, nCD64 showed the highest overall diagnostic accuracy. Its AUC of 0.816 was slightly higher than that of NLR (0.804), TLC (0.781), procalcitonin (0.719), hs-CRP (0.577) and ESR (0.463). However, these descriptive AUC differences should not automatically be interpreted as statistically significant between-test differences because a formal paired ROC comparison, such as DeLong's test, was not reported.

The total leukocyte count was significantly higher in infected patients than in those with sterile inflammation (15,620±5,840 versus 10,980±4,120 cells/mm³; $p<0.001$). At ≥13,200 cells/mm³, TLC showed 73.5% sensitivity, 75.8% specificity and an AUC of 0.781. Although useful, leukocytosis is a characteristic feature of alcoholic hepatitis itself and may be influenced by corticosteroids, physiological stress, gastrointestinal bleeding and hepatic inflammation. Pandey et al. (2021)[1] reported a considerably lower TLC AUC of 0.63, supporting the limited disease-specificity of leukocyte count.

NLR was also significantly elevated in the infection group (9.15±7.10 versus 5.28±4.20; $p=0.009$). At ≥5.8, it demonstrated 79.4% sensitivity, 81.8% specificity and 80.6% accuracy, with an AUC of 0.804. Forrest et al. (2019)[14] reported that baseline NLR was associated with infection, renal dysfunction and corticosteroid response in alcoholic hepatitis, supporting its role as a readily available marker of systemic inflammatory severity. Kwon et al. (2015)[15] also found NLR useful in hospitalized patients with cirrhosis, particularly for prognostic assessment. However, the NLR may increase in both bacterial infection and sterile inflammation; therefore, its apparently strong performance in the present study requires validation in a larger cohort.

Serum procalcitonin showed the highest sensitivity among the conventional biomarkers at 82.4%, but specificity was only 60.6%, producing an AUC of 0.719. Kang et al. (2024)[16] prospectively evaluated 108 patients with severe alcoholic hepatitis, of whom 31 (28.7%) had bacterial infection. Median PCT was higher in infected than non-infected patients (1.5 versus 0.4 ng/mL), and the AUC for bacterial infection was 0.752, compared with 0.655 for CRP. For sepsis, PCT significantly outperformed CRP, with AUCs of 0.780 and 0.630, respectively. These results were close to the present PCT AUC of 0.719 and hs-CRP AUC of 0.577.

Pandey et al. (2021)[1] reported better PCT performance, with an AUC of 0.86, sensitivity of 83% and specificity of 72% at a cut-off of 0.261 ng/mL. Differences from the present results may reflect the higher ≥0.50 ng/mL cut-off, assay variation and the markedly right-skewed PCT distribution. Rule et al. (2015)[17] demonstrated that procalcitonin may

increase in severe hepatocellular injury even without bacterial infection, indicating that PCT can partly reflect cell injury rather than infection alone. This may explain the reduced specificity observed in severe alcoholic hepatitis.

The mean hs-CRP concentrations did not differ significantly between groups (62.06±36.06 versus 55.44±49.98 mg/L; p=0.538). Its AUC of 0.577, sensitivity of 67.6% and specificity of 54.5% indicated poor discrimination. Kang et al. (2024)[16] similarly found CRP inferior to PCT in severe alcoholic hepatitis. Pérez-Hernández et al. (2024)[18] also emphasized the difficulty of diagnosing infection using conventional inflammatory and oxidative-stress markers in severe alcoholic hepatitis because intense sterile inflammation may generate substantial biomarker elevation. Moreover, impaired hepatic synthetic function may cause inconsistent CRP responses, further limiting its reliability.

ESR performed least effectively, with no significant difference between infected and non-infected patients and an AUC of 0.463. ESR is affected by anaemia, immunoglobulin concentrations, plasma proteins and advanced liver disease and is therefore poorly suited for identifying acute bacterial infection in this population. Yeh et al. (2019)[12] and Cong et al. (2021)[9] likewise supported the superiority of nCD64 and procalcitonin over nonspecific inflammatory markers.

Overall, the findings show that nCD64 provides the best combination of specificity and accuracy for distinguishing bacterial infection from sterile inflammation in severe alcoholic hepatitis with SIRS. NLR and TLC may be useful, inexpensive adjuncts, while procalcitonin offers relatively high sensitivity but lower specificity. Combining nCD64 with clinical evaluation, cultures and procalcitonin may improve sensitivity; Pandey et al. (2021)[1] reported that considering either elevated nCD64 or elevated PCT as a positive result increased sensitivity to 98.3%, although specificity declined. Future studies should validate combined algorithms, standardize flow-cytometric reporting and assess whether nCD64-guided decisions reduce unnecessary antibiotics or permit safer initiation of corticosteroids. Recent evidence from Kulkarni et al. (2025)[19] further demonstrates that infection remains a major treatment-related concern in severe alcohol-associated hepatitis, reinforcing the clinical relevance of rapid and reliable infection biomarkers.

CONCLUSION

Quantitative neutrophil CD64 expression was effective in distinguishing bacterial infection from sterile inflammation among patients with severe alcoholic hepatitis satisfying SIRS criteria. The mean percentage of nCD64-expressing neutrophils was significantly higher in patients with bacterial infection than in those with sterile inflammation (46.27±33.12% versus 11.75±16.37%; p<0.001). At an optimal cut-off of ≥27%, nCD64 demonstrated 76.5% sensitivity, 90.9% specificity, 89.7% positive predictive value, 78.9% negative predictive value and 83.6% overall diagnostic accuracy. Its AUC of 0.816 confirmed good discriminatory ability. Patients with nCD64 ≥27% had 32.50 times higher odds of bacterial infection than those with lower expression. Among the evaluated biomarkers, nCD64 showed the highest specificity and overall accuracy, outperforming serum procalcitonin, hs-CRP, ESR and total leukocyte count and showing slightly better discrimination than the neutrophil-to-lymphocyte ratio. Therefore, nCD64 may serve as a useful adjunct to clinical evaluation, microbiological cultures and radiological investigations for the early identification of bacterial infection in severe alcoholic hepatitis with SIRS. It should, however, not be used as a standalone replacement for comprehensive infection assessment.

LIMITATIONS OF THE STUDY

1. **Small sample size:** The study included only 67 patients, comprising 34 with bacterial infection and 33 with sterile inflammation. This limited the precision of diagnostic estimates, as reflected by the relatively wide confidence intervals.
2. **Single-centre study:** The study was conducted at one tertiary-care hospital; therefore, its findings may not be generalizable to other institutions, geographical regions or patient populations.
3. **Observational design:** The prospective observational design allowed assessment of diagnostic associations but could not establish whether nCD64-guided clinical management improved patient outcomes.
4. **Potential misclassification of infection:** Some patients might have had clinically important culture-negative infections. Previous antibiotic exposure could have reduced culture yield and resulted in misclassification between infection and sterile-inflammation groups.
5. **Heterogeneous infection definition:** Proven and probable bacterial infections were analysed together. Although clinically appropriate, combining microbiologically confirmed and clinically diagnosed infections might have introduced diagnostic heterogeneity.
6. **Single-time-point measurement:** Neutrophil CD64 and other biomarkers were measured principally at enrolment. Serial

changes during antibiotic or corticosteroid treatment were not assessed.

7. **Lack of assay standardization:** CD64 expression may vary with the flow cytometer, antibody clone, gating strategy, sample-processing time and reporting method. Consequently, the ≥27% cut-off requires external laboratory validation.
8. **Non-significant fluorescence-intensity measurements:** Although nCD64 percentage differed significantly, mean and median fluorescence-intensity measurements did not. This discrepancy requires confirmation using standardized CD64 indices.
9. **Skewed biomarker distributions:** Serum procalcitonin showed substantial variability and right-skewness. Analysis based on mean and standard deviation might not have adequately represented its distribution; median and interquartile range with nonparametric testing would have been preferable.
10. **No formal comparison of ROC curves:** The AUCs of different biomarkers were described, but formal paired comparisons using methods such as the DeLong test were not performed. Therefore, statistical superiority of nCD64 over individual biomarkers could not be conclusively established.
11. **Potential confounding:** Disease severity, renal dysfunction, prior antibiotics, corticosteroid exposure and infection site might have affected biomarker concentrations. Multivariable adjustment was limited by the small sample size.
12. **Clinical outcomes were not evaluated:** The study did not determine whether nCD64 predicted mortality, organ failure, length of hospital stay, response to antibiotics or eligibility and response to corticosteroid treatment.

REFERENCES

1. Pandey G, Singh H, Chaturvedi S, Hatti M, Kumar A, Mishra R, et al. Utility of neutrophil CD64 in distinguishing bacterial infection from inflammation in severe alcoholic hepatitis fulfilling SIRS criteria. Sci Rep. 2021;11(1):19726. doi:10.1038/s41598-021-99276-y.
2. Karakike E, Moreno C, Gustot T. Infections in severe alcoholic hepatitis. Ann Gastroenterol. 2017;30(2):152–160. doi:10.20524/aog.2016.0101.
3. Michelena J, Altamirano J, Abraldes JG, Affò S, Morales-Ibanez O, Sancho-Bru P, et al. Systemic inflammatory response and serum lipopolysaccharide levels predict multiple organ failure and death in alcoholic hepatitis. Hepatology. 2015;62(3):762–772. doi:10.1002/hep.27779.
4. Maiwall R, Chandel SS, Wani Z, Kumar S, Sarin SK. SIRS at admission is a predictor of AKI development and mortality in hospitalized patients with severe alcoholic hepatitis. Dig Dis Sci. 2016;61(3):920–929. doi:10.1007/s10620-015-3921-4.
5. Thursz MR, Richardson P, Allison M, Austin A, Bowers M, Day CP, et al. Prednisolone or pentoxifylline for alcoholic hepatitis. N Engl J Med. 2015;372(17):1619–1628. doi:10.1056/NEJMoa1412278.
6. Vergis N, Atkinson SR, Knapp S, Maurice J, Allison M, Austin A, et al. In patients with severe alcoholic hepatitis, prednisolone increases susceptibility to infection and infection-related mortality, and is associated with high circulating levels of bacterial DNA. Gastroenterology. 2017;152(5):1068–1077.e4. doi:10.1053/j.gastro.2016.12.019.
7. Jophlin LL, Singal AK, Bataller R, Wong RJ, Sauer BG, Terrault NA, et al. ACG clinical guideline: alcohol-associated liver disease. Am J Gastroenterol. 2024;119(1):30–54. doi:10.14309/ajg.0000000000002572.
8. Wang X, Li ZY, Zeng L, Zhang AQ, Pan W, Gu W, et al. Neutrophil CD64 expression as a diagnostic marker for sepsis in adult patients: a meta-analysis. Crit Care. 2015;19:245. doi:10.1186/s13054-015-0972-z.
9. Cong S, Ma T, Di X, Tian C, Zhao M, Wang K. Diagnostic value of neutrophil CD64, procalcitonin and interleukin-6 in sepsis: a meta-analysis. BMC Infect Dis. 2021;21(1):384. doi:10.1186/s12879-021-06064-0.
10. Patnaik R, Azim A, Agarwal V. Neutrophil CD64: a diagnostic and prognostic marker of sepsis in adult critically ill patients a brief review. Indian J Crit Care Med. 2020;24(12):1242–1250. doi:10.5005/jp-journals-10071-23558.
11. Pham HM, Nguyen HT, Tran QH, Nguyen THT. Diagnostic value of neutrophil CD64 in sepsis patients in the intensive care unit: a cross-sectional study. Diagnostics (Basel). 2023;13(8):1427. doi:10.3390/diagnostics13081427.
12. Yeh CF, Wu CC, Liu SH, Chen KF. Comparison of the accuracy of neutrophil CD64, procalcitonin and C-reactive protein for sepsis identification: a systematic review

and meta-analysis. Ann Intensive Care. 2019;9(1):5. doi:10.1186/s13613-018-0479-2.

13. Shi J, Tang J, Chen D. Meta-analysis of diagnostic accuracy of neutrophil CD64 for neonatal sepsis. Ital J Pediatr. 2016;42:57. doi:10.1186/s13052-016-0268-1.
14. Forrest EH, Storey N, Sinha R, Atkinson SR, Vergis N, Richardson P, et al. Baseline neutrophil-to-lymphocyte ratio predicts response to corticosteroids and is associated with infection and renal dysfunction in alcoholic hepatitis. Aliment Pharmacol Ther. 2019;50(4):442–453. doi:10.1111/apt.15335.
15. Kwon JH, Jang JW, Kim YW, Lee SW, Nam SW, Jaegal D, et al. The usefulness of C-reactive protein and neutrophil-to-lymphocyte ratio for predicting the outcome in hospitalized patients with liver cirrhosis. BMC Gastroenterol. 2015;15:146. doi:10.1186/s12876-015-0378-z.
16. Kang MK, Lee YR, Park SY, Heo NY, et al. Diagnostic performance of procalcitonin for bacterial infection in severe alcoholic hepatitis compared with C-reactive protein. BMC Gastroenterol. 2024;24:428. doi:10.1186/s12876-024-03519-x.
17. Rule JA, Hynan LS, Attar N, Sanders C, Korzun WJ, Lee WM, et al. Procalcitonin identifies cell injury, not bacterial infection, in acute liver failure. PLoS One. 2015;10(9):e0138566. doi:10.1371/journal.pone.0138566.
18. Pérez-Hernández O, González-Reimers E, García-Rodríguez A, et al. Value of inflammatory response and oxidative damage in the diagnosis of infections in severe alcoholic hepatitis. Eur J Intern Med. 2024;119:64–70. doi:10.1016/j.ejim.2023.08.005.
19. Kulkarni AV, Arab JP, Premkumar M, et al. Infections in standard or tapered dose of prednisolone for severe alcohol-associated hepatitis: a randomized controlled trial. Am J Gastroenterol. 2025;120:1087–1097.