

## Original Research Article

# Evaluation of eosinophil count and neutrophil-lymphocyte count ratio versus C-reactive protein levels in patients with sepsis

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### ABSTRACT

**Background:** One major problem encountered in the intensive care unit is differentiating the inflammatory response from an infective process. Clinical and standard laboratory tests are not very helpful because most critically ill patients develop some degree of inflammatory response, whether or not they have sepsis. Numerous biomarkers have been evaluated to predict mortality in critically ill patients, although none have proved entirely useful. Objective of the study was to evaluate eosinophil count and neutrophil-lymphocyte count ratio with C-reactive protein levels in patients with sepsis.

**Methods:** 71 patients >18 years of age of either sex with a diagnosis of sepsis were enrolled in this one-year observational study. Patients were classified according to the criteria of the American College of Chest Physicians/Society of Critical Care Medicine into sepsis group (n=50) and no sepsis group (n=21). Sepsis group were further divided into subgroups: sepsis (n=19), severe sepsis (n=16) and septic shock (n=15). Absolute eosinophil cell, neutrophil and lymphocyte counts for first 4 consecutive days and then on alternate days up to one week were also noted down. C-reactive protein levels on day 3 were also noted down.

**Results:** In the sepsis group, mean eosinophil count was significantly ( $p<0.0001$ ) low, mean neutrophil/lymphocyte count ratio was significantly ( $p<0.0001$ ) high, mean CRP count was significantly ( $p=0.019$ ) more as compared to that of no sepsis group. Among 16 mortalities, significant ( $p<0.05$ ) decrease was noted in mean eosinophil count from day 3 onwards in patients of sepsis and septic shock subgroups. Mean N/L ratio showed no significant difference in patients of sepsis, severe sepsis or septic shock. Mean CRP count showed significant ( $p<0.05$ ) increase in severe sepsis patients and mean Apache II score showed significant ( $p<0.05$ ) deterioration in patients of septic shock.

**Conclusions:** Neutrophil/lymphocyte count ratio (NLCR) and absolute eosinophil count (AEC) came out as better independent biomarker of sepsis in critically ill patients with infection admitted in intensive care unit. Diagnostic performance was better in these two diagnostic markers as compared to CRP marker. NLCR presented with sensitivity of 89.58%, AEC with 82.35% and CRP with 80.77%. Outcomes of NLCR and AEC were quick, easy and economical in establishing diagnosis of sepsis.

**Keywords:** Absolute eosinophil count, C-reactive protein, Neutrophil/lymphocyte count ratio, Intensive care unit, Sepsis

### INTRODUCTION

One of the most frequent problems in the intensive care unit is actually differentiating the inflammatory response

from an infective process.<sup>1</sup> Sepsis is known as the clinical syndrome resulting from the presence of both infection and a systemic inflammatory response. It involves the activation of inflammatory and anti-inflammatory

mediators, cellular and humoral reactions, and micro- and macro-circulatory alterations.<sup>2,3</sup>

The definition of sepsis requires the presence of infection and at least two signs of systemic inflammation. Severe sepsis is defined when sepsis results in dysfunction of at least one remote organ function. Septic shock is defined as sepsis with hypotension (systolic blood pressure of <90 mm Hg or a reduction of 40 mm Hg from baseline) despite adequate fluid resuscitation. Concomitant organ dysfunction or perfusion abnormalities (e.g., lactic acidosis, oliguria, or coma) are present in the absence of other known causes.<sup>4</sup>

Early diagnosis of sepsis is vital because rapid, appropriate therapy is associated with improved outcomes.<sup>5</sup> The two biomarkers that have been most widely studied and used in patients with sepsis are C-reactive protein (CRP) and procalcitonin (PCT). Levels of both these biomarkers have been demonstrated to be raised in patients with sepsis making them useful diagnostic indicators. However, because they lack specificity for sepsis and levels may be raised in other inflammatory diseases, these biomarkers are more useful for ruling out sepsis than for ruling it in, that is, a completely normal value makes a diagnosis of sepsis very unlikely.<sup>6</sup>

Eosinopenia is an attractive potential biomarker in sepsis, as the eosinophil count is already serially measured in routine clinical practice and the additional costs would therefore be minimal.<sup>7</sup> Eosinopenia has also been proposed as a marker that may help to differentiate sepsis-related conditions from other causes of SIRS.<sup>8</sup> The usefulness of eosinopenia as predictor of outcome in critically ill patients has also been reported.<sup>9</sup>

The total leukocyte and neutrophil counts have historically been used as markers of infection. An association was found between infection and monocyte and lymphocyte counts, as well as specific associations between these two counts.<sup>10</sup> The neutrophil-lymphocyte ratio (NLR) is a simple biomarker of inflammation. Several studies have reported that an elevated NLR (in the peripheral blood) is associated with a poor prognosis.<sup>11</sup>

The present prospective, observational study was undertaken to assess and compare eosinophil count, neutrophil-lymphocyte count ratio with C-reactive protein in patients admitted in the medicine intensive care unit with classification of sepsis according to the criteria of American College of Chest Physicians/Society of Critical Care Medicine.<sup>12</sup>

## METHODS

This comparative study was conducted on 71 patients >18 years of age of either sex with a diagnosis of sepsis admitted to the Medicine Intensive Care Unit of Acharya

Shri Chander College of Medical Science and Hospital, Sidhra, Jammu. Patients who died or were discharged within 72 hours of admission, with hematological cancer, HIV infection, bronchial asthma, hay fever, atopic dermatitis, allergic conjunctivitis, trauma, myocardial infarction, rheumatoid arthritis and patients who underwent chemotherapy, glucocorticoid medication and postoperative surgical and burn patients were excluded from the study. Patients were classified according to the criteria of the American College of Chest Physicians/Society of Critical Care Medicine (12) into sepsis group (n=50) and no sepsis group (n=21). Sepsis group were further divided into subgroups: sepsis (n=19), severe sepsis (n=16) and septic shock (n=15).

Systemic inflammatory response syndrome (SIRS) was defined by two or more of the following criteria: body temperature >38°C or <36°C, heart rate >90 beats/min, respiratory rate >20/min or PaCO<sub>2</sub> < 32 Torr, and white blood cell count >12,000 cells/mm<sup>3</sup>, <4,000 cells/mm<sup>3</sup>, or >10% immature forms. Sepsis is a SIRS associated with the presence of an infectious process. Severe sepsis is a sepsis associated with organ dysfunction, hypoperfusion, or hypotension (systolic blood pressure <90 mmHg or a reduction ≥ 40 mmHg from baseline). Septic shock is a subset of severe sepsis and is defined as a persisting sepsis-induced hypotension despite adequate fluid resuscitation.

Infection was diagnosed by textbook standard criteria (13) and was categorized according to the following: culture/microscopy of a pathogen from a clinical focus; positive urine dip test in the presence of dysuria symptoms; clinical lower respiratory tract symptoms and radiographic pulmonary abnormalities that are at least segmental and not due to pre-existing or other known causes; infection documented with another imaging technique; lumbar puncture when meningitis was suspected; obvious clinical infection (erysipelas); and identification of a pathogen by serology or by PCR.

The study protocol was approved by the hospital ethics committee. Informed written consent was taken from all the enrolled patients. At the time of ICU admission, for each patient age, gender and provisional diagnosis, and vital signs (body temperature, heart rate, respiratory rate, systolic and diastolic arterial pressure, and urine rate) were recorded in the predesigned proforma. The Acute Physiology and Chronic Health Evaluation (APACHE) II score (14) was calculated on admission.

Blood samples were obtained by venipuncture on admission, and subsequently each morning. The white blood cell count, absolute eosinophil cell count, neutrophil-lymphocyte cell count ratio and the C-reactive protein (CRP) level based on turbidimetry were recorded on admission to the ICU. Automated Haematology Cell Counter (Melet Schloesing Laboratories) was used for analyzing of blood sample. Absolute eosinophil cell, neutrophil and lymphocyte counts for first 4 consecutive

days and then on alternate days up to one week were also noted down. C-reactive protein levels on day 3 were also noted down in the proforma.

Data was presented as mean ± standard deviation for variables with a normal distribution. Statistical differences between groups were evaluated by the chi-square test for categorical variables. Comparison of group differences for continuous variables was carried out by one-way analysis of variance (ANOVA). Bonferroni's post hoc test was used to find out intragroup significance. The sensitivity, specificity, and positive and negative likelihood ratios [with 95% confidence intervals (CIs)] was calculated at the best cutoff value. A two-tailed p value of <0.05 was considered statistically significant.

**RESULTS**

Out of 71 patients enrolled in the study, 50 were diagnosed with sepsis according to the criteria of American College of Chest Physicians/Society of Critical Care Medicine. Mean age (± standard deviation) of patients in the sepsis group 54.22 (± 8.08) years was comparable (p=0.61) to that of patients in the no sepsis group 53.19 (± 7.65) years. In the sepsis group, there were 23 (46%) male and 27 (54%) female patients, while in no sepsis group there were 7 (33.33%) male and 14 (66.67%) female patients. Statistically, there was no significant difference between the two groups (p=0.07). In the sepsis group, mean eosinophil count was significantly (p<0.0001) low as compared to that of no sepsis group (27.43 vs 152.42). Mean neutrophil/lymphocyte ratio in sepsis group was significantly (p<0.0001) high as compared to that of no sepsis group (9.47 vs 2.82). Mean CRP count of sepsis group was significantly (p=0.019) more than that of no sepsis (62.10 vs 45.85). Mean Apach II score in sepsis group was significantly (p<0.0001) more than that of no sepsis group (21.68 vs 11.57).

**Table 1: Diagnostic value of eosinophil count at 50 cells/mm<sup>3</sup> cutoff point.**

| Diagnostic value                | Value  | 95% confidence interval |
|---------------------------------|--------|-------------------------|
| Sensitivity                     | 82.35% | 69.43 – 90.57           |
| Specificity                     | 60.00% | 38.61 – 78.06           |
| Positive likelihood ratio (PV+) | 2.06   | 1.19 – 3.57             |
| Negative likelihood ratio (PV-) | 0.29   | 0.15 – 0.59             |
| Positive Predictive Value (PPV) | 0.840  | 0.738 – 0.942           |
| Negative Predictive Value (NPV) | 0.571  | 0.359 – 0.783           |
| Relative risk                   | 1.96   | 1.208 – 3.19            |
| Odds ratio                      | 7.00   | 2.28 – 21.45            |
| Area under curve (AUC)          | 0.712  | -                       |
| Accuracy                        | 76.06  | -                       |

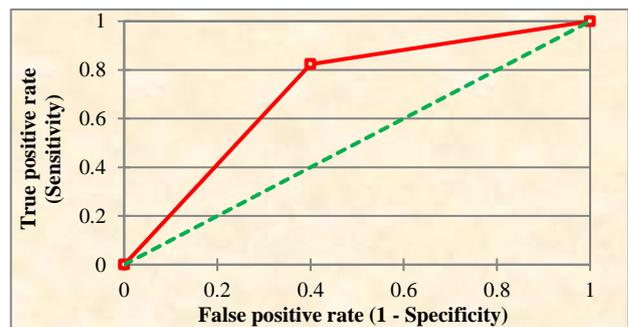
Diagnostic value of eosinophil count at 50 cells/mm<sup>3</sup> cutoff point, N/L ratio at <5 cutoff point and CRP count at <50 mg/dL cutoff point is given in Tables 1, 2 and 3 (Figure. 1, 2 and 3) respectively.

**Table 2: Diagnostic value of N/L ratio at <5 cutoff point.**

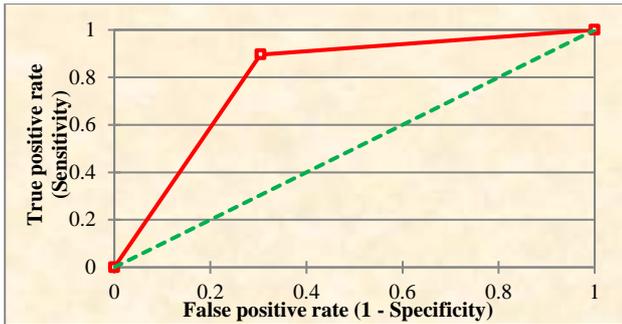
| Diagnostic value                | Value  | 95% confidence interval |
|---------------------------------|--------|-------------------------|
| Sensitivity                     | 89.58% | 77.26 – 95.82           |
| Specificity                     | 69.57% | 48.89 – 84.45           |
| Positive likelihood ratio (PV+) | 2.94   | 1.58 – 5.50             |
| Negative likelihood ratio (PV-) | 0.15   | 0.06 – 0.36             |
| Positive Predictive Value (PPV) | 0.860  | 0.764 – 0.956           |
| Negative Predictive Value (NPV) | 0.762  | 0.589 – 0.944           |
| Relative risk                   | 3.61   | 1.74 – 7.49             |
| Odds ratio                      | 19.66  | 5.70 – 67.77            |
| Area under curve (AUC)          | 0.796  | -                       |
| Accuracy                        | 83.09  | -                       |

**Table 3: Diagnostic value of CRP count at <50 mg/dl cutoff point.**

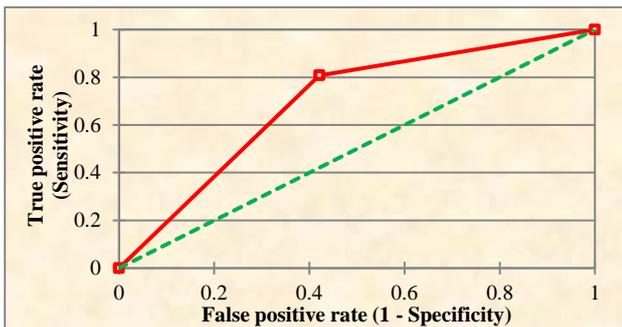
| Diagnostic value                | Value  | 95% confidence interval |
|---------------------------------|--------|-------------------------|
| Sensitivity                     | 80.77% | 67.82 – 89.32           |
| Specificity                     | 57.89% | 36.26 – 76.78           |
| Positive likelihood ratio (PV+) | 1.92   | 1.11 – 3.03             |
| Negative likelihood ratio (PV-) | 0.33   | 0.17 – 0.65             |
| Positive Predictive Value (PPV) | 0.840  | 0.738 – 0.942           |
| Negative Predictive Value (NPV) | 0.524  | 0.310 – 0.737           |
| Relative risk                   | 1.76   | 1.13 – 2.76             |
| Odds ratio                      | 5.77   | 1.89 – 17.60            |
| Area under curve (AUC)          | 0.693  | -                       |
| Accuracy                        | 74.65  | -                       |



**Figure 1: ROC curve of eosinophil count.**



**Figure 2: ROC curve of N/L ratio.**



**Figure 3: ROC curve of CRP count.**

Out of 50 patients in the sepsis group, 16 (32%) died during 7 days under observation. Most mortalities 6 (40%) were observed in patients with septic shock (n=15), followed by 5 (31.25%) in patients with severe sepsis (n=16) and 5 (26.32%) in patients with sepsis (n=19). Among 34 survival patients, significant (p<0.05) increase was noted in mean eosinophil count from day 3 onwards in patients of sepsis, severe sepsis and septic shock subgroups. Mean N/L ratio showed significant (p<0.05) decreasing trend in patients of severe sepsis and septic shock.

Mean CRP count showed significant (p<0.05) decline in septic shock patients and mean Apache II score showed significant (p<0.05) improvement in patients of severe sepsis. Among 16 mortalities, significant (p<0.05) decrease was noted in mean eosinophil count from day 3 onwards in patients of sepsis and septic shock subgroups. Mean N/L ratio showed no significant difference in patients of sepsis, severe sepsis or septic shock. Mean CRP count showed significant (p<0.05) increase in severe sepsis patients and mean Apache II score showed significant (p<0.05) deterioration in patients of septic shock.

In the infected group (n=50), 26 (52%) patients were diagnosed with respiratory tract infection, 16 (32%) with urinary tract infection, and 4 (8%) patients each with diabetic foot and high systolic blood pressure. Among patients in non-infected group (n=21), 10 (47.62%) patients had acute ischemic stroke, 3 (14.29%) each had seizure, acute renal failure and congestive cardiac failure, while 2 (9.52%) patients had hypercalcemia.

## DISCUSSION

Sepsis is one of the leading causes of death in critically ill patients. Because sepsis has a high prevalence worldwide with high morbidity and mortality rates, standardizing the diagnostic criteria for early recognition of the syndrome is essential. To be considered a valid biomarker, three aspects must be present: (i) proving that the test truly measures a particular molecular species or its relevant biological activity; (ii) proving that measurement of the biomarker discriminates patients with a disease from those who are without the disease; (iii) proving that measurement of the biomarker can inform a clinical decision that can improve patient outcomes.<sup>15</sup>

Eosinophils normally account for only 1 to 3% of peripheral blood leucocytes, and the upper limit of the normal range is 350 cells/mm<sup>3</sup>.<sup>16</sup> Mechanisms that control eosinopenia in acute infection, also considered as an acute stress, involve mediation by adrenal glucocorticosteroids and epinephrine.<sup>17</sup> As a cheap test to diagnose sepsis on ICU admission, eosinopenia offers a higher degree of certainty than other currently available tests or markers.<sup>8</sup>

In the present study, eosinophil count 50 cells/mm<sup>3</sup> was taken as a cut-off point for judging of severity in the patients of sepsis. Gil et al showed that an eosinophil count <40/mm<sup>3</sup> was strongly related to the presence of bacterial infections.<sup>18</sup> In our study, mean eosinophil count significantly improved from day 1 to day 3 in 34 patients who survived. In sepsis group (n=14), eosinophil count increased significantly from 35.76 to 56.69 (p=0.000), in severe sepsis group (n=11, it increased significantly from 20.88 to 65.08 (p=0.001) and in septic shock group, eosinophil count increased significantly from 22.48 to 59.28 (p=0.000). In the mortal group (n=16), eosinophil count decreased significantly from 32.29 to 20.17 in sepsis (p=0.04), from 33.90 to 21.72 in severe sepsis group (p=0.21) and decreased significantly from 26.24 to 17.30 in septic shock group (p=0.02). Abidi et al also reported weak but significant correlation with sepsis parameters and with the severity of the disease.<sup>8</sup> Holland et al also suggested that eosinophil count could be a useful marker of severity and prognosis independently of other routinely used indicators.<sup>19</sup>

In our study, eosinophil count of 50 cells/mm<sup>3</sup> had a sensitivity of 82.35% with CI of 69.43-90.57%, specificity of 60% with CI of 38.61-78.06%, positive likelihood ratio (PV+) of 2.06 with CI of 1.19-3.57 and negative likelihood ratio (PV-) of 0.29 with CI of 0.15-0.59. Abidi et al. (8), with a cut-off value of 50 cells/mm<sup>3</sup>, reported a sensitivity of 80% (95% CI, 71 to 86%), a specificity of 91% (95% CI, 79 to 96%), a positive likelihood ratio of 9.12 (95% CI, 3.9 to 21) and a negative likelihood ratio of 0.21 (95% CI, 0.15 to 0.31). Shaaban et al reported that in a total of 68 patients enrolled into a study in a critical care unit, eosinophil cell count, with cut-off of 50 cells/mm<sup>3</sup>, produced a

sensitivity of 81%, specificity of 65%, a PPV of 66% and NPV of 80%.<sup>20</sup>

Evidence is growing that the neutrophil/lymphocyte count ratio (NLCR) is useful in the prediction of survival in various clinical settings. In the present study of 50 patients with diagnosis of sepsis and 21 patients with no sepsis NLR  $\geq 5$  was used for the diagnosis of patients with sepsis in accordance with the study of Gurol et al, who reported that an NLR value  $\geq 5$  may be a more convenient marker than CRP, due to its improved ability to detect bacterial infections at lower cost.<sup>21</sup>

The value of NLCR increased from  $8.36 \pm 1.04$  to  $8.72 \pm 1.01$  in sepsis group (p-value 0.592), from  $11.32 \pm 1.55$  to  $15.39 \pm 5.16$  (p-value of 0.155) in severe sepsis group and from  $14.70 \pm 5.81$  to  $15.20 \pm 6.32$  (p-value 0.863) in septic shock group. Also in the patients who survived value of NLCR on day 3 decreased with decreasing severity of sepsis after the initiation of therapy. These values correlated with the values of APACHE 2 which showed similar pattern as of NLCR. So, it was seen that NLCR could predict the diagnosis of sepsis as well as the severity of sepsis. This was in accordance with the study of de Jager et al who investigated and found significant differences between patients with positive and negative blood cultures were detected with respect to the CRP level, lymphocyte count and NLCR.<sup>10</sup> They concluded that in an emergency care setting, both lymphocytopenia and NLCR are better predictors of bacteremia than routine parameters like CRP level, WBC count and neutrophil count. Attention to these markers is easy to integrate in daily practice and without extra costs.

In our study NLR of  $\geq 5$  had a sensitivity of 89.58% with CI of 77.26-95.82, specificity of 69.57% with CI of 48.89-84.45, negative likelihood ratio (PV-) of 0.15 with CI of 0.06-0.36 and positive likelihood ratio (PV+) of 2.94 with CI of 1.58-5.50. Terradas et al study indicated that a neutrophil cell count to lymphocyte count ratio of below 7 was indicative of a good outcome giving sensitivities of 40.91%, specificities of 93.22%, negative likelihood ratio of 0.63 and positive likelihood ratio of 6.03. Okyay et al concluded that NLCR is an easy and inexpensive laboratory measure and might provide significant information regarding inflammation in chronic kidney disease including pre-dialysis and dialysis patients.<sup>22,23</sup>

C-reactive protein is a long-established marker of sepsis. C-reactive protein belongs to the pentraxin family of proteins, so called because they form a cyclic pentamer composed of five identical non-glycosylated sub-units, non-covalently bound and organised in a very stable discoid-like structure. CRP rises whenever an inflammatory process is present. The serum concentration of CRP in the normal human population has a median of 0.8 mg/l and is below 10 mg/l in 99% of normal samples.<sup>24</sup> Levels above these values are abnormal and indicate the presence of a disease process. CRP level is

independent of the underlying pathology and is not modified by any therapy or intervention such as renal replacement therapy

In the present study CRP of 50 mg/dl was used as a cut-off value between patients with sepsis and non-sepsis. In our study, CRP of 50 mg/dl had a sensitivity of 77.50% (C.I. of 62.21-87.79), specificity of 58.06% (C.I. of 40.75-73.54) and PPV of 0.705 (C.I. of 0.569-0.839) in diagnosis of CRP. This value is in accordance with the study of Povoia et al who concluded that a plasma CRP of 50 mg/l or more was highly suggestive of sepsis with sensitivity 98.5% and specificity of 75%.<sup>25</sup>

CRP was not only useful in the diagnosis but also related with the severity. The value of CRP increased with the severity of sepsis. In our study mean CRP value in sepsis was 49.46 and in severe sepsis mean value WAS 69.40. So, a statistically significant co-relation was seen between sepsis and severe sepsis. Also in septic shock the value of CRP increased further to a mean of 72.83. But this difference was however not statistically significant.

After the diagnosis of sepsis CRP levels at day 1 and day 3 were measured and two types of pattern were seen. After therapy was started the CRP levels as well as the severity of sepsis decreased on day 3 in all patients who survived whereas patients who died during their stay in ICU their CRP levels on day 1 and day 3 increased from baseline of  $72.68 \pm 17.38$  to  $85.38 \pm 13.83$  (p-value of 0.238) in the sepsis group, from  $72.31 \pm 10.83$  to  $88.04 \pm 5.83$  (p-value of 0.028) in severe sepsis group and from  $87.33 \pm 7.53$  to  $102.12 \pm 7.049$  (p-value of 0.050) in septic shock group respectively. It was also observed that CRP in various groups were in accordance with the value of APACHE 2 score whose value decreased with decreasing severity of sepsis and increased on day 3 in patients who died.

In the present study, among infected patients (n=50), 16 (32%) died during the course of one week. All these patients had high neutrophil/lymphocyte ratio, decreased eosinophil count, high CRP and high Apache II score from the day 3 onwards. Five (31.25%) patients each had either sepsis or severe sepsis, while 6 (37.50%) patients had septic shock. Povoia et al reported mortality of 38% in their cohort of 891 community-acquired sepsis patients admitted to intensive care unit.<sup>26</sup> Chang et al in their study reported 20% death in sepsis group, 40% in severe sepsis and 60% in septic shock group.<sup>27</sup>

## CONCLUSION

In the present study, neutrophil/lymphocyte count ratio (NLCR) and absolute eosinophil count (AEC) came out as better independent biomarker of sepsis in critically ill patients with infection admitted in intensive care unit. Diagnostic performance was better in these two diagnostic markers as compared to CRP marker. NLCR presented with sensitivity of 89.58%, AEC with 82.35%

and CRP with 80.77%. Outcomes of NLCR and AEC were quick, easy and economical in establishing diagnosis of sepsis. These markers can be used on regular basis in clinical practice.

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