Diagnostic Value of Eosinopenia and Neutrophil to Lymphocyte Ratio in Early Onset Neonatal Sepsis

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Abstract:

Background: Neonatal sepsis remains a challenge for neonatal care providers. The accurate and timely diagnosis of neonatal sepsis remains a challenging issue due to its non-specific clinical presentation.

Patients and Methods: This descriptive cross sectional study was carried out at neonatal intensive care unit in Fayoum University Hospital from February to December 2019. Samples were obtained from 100 neonates that were met the inclusion criteria as having EONS. Neonates were subjected to: History taking, through clinical examination for manifestations of sepsis. Complete blood count, differential leucocytic count, C-reactive protein and Blood culture were done to all neonates.

Results: of 100 neonates who met the inclusion criteria, (61%) were males and (39%) were females.71 (71%) subjects were included in confirmed sepsis group and 29 (29%) were in non-confirmed sepsis group. Mean eosinophil count in confirmed EONS and non- confirmed EONS group were 250 ± 170 cells/mm3 and 670 ± 470 cells/mm3 , respectively, considered as significant (p<0.001).Result

from diagnostic test of eosinopenia from the EONS group (cut-off point 280

cells/mm3) obtained 56.3% sensitivity and 93.1% specificity. Mean NLR in EONS and non-confirmed EONS group were 3.08 ± 1.9 and 1.29 ± 0.76, respectively, also considered as significant (p<0.001).Result for diagnostic test of NLR in EONS group (cut-off point 1.75) obtained 70.4% sensitivity and 75.9% specificity.

Conclusion: Eosinopenia and increased in NLR both have a high specificity value as a diagnostic marker of EONS.

Key words: Eosinophil count, Neutrophil to lymphocyte ratio, Early onset neonatal sepsis.

Introduction:

Neonatal sepsis is a clinical syndrome arising from the invasion of microorganisms into the blood stream that arises in the first month of life (1).Neonatal sepsis is a major cause of mortality in the developing countries. As the signs and symptoms of sepsis are non-specific, early diagnosis poses a challenge to the clinicians (2)**.**Neonatal sepsis is still a major problem in neonatal care and still

contributes significantly to disability and death. At least 1 million deaths occuring in the newborn period (0-28 days) per year are caused by infection, of which nearly 25% is the cause of newborn mortality and accounting for 10% of infant mortality worldwide (3)**.**

Evidence of infection from blood cultures itself often show insignificant results and takes too much time to obtain (4)**.**Therefore, other test, such as a complete blood count (CBC) is done along with the blood culture to determine sepsis. However even with current severity and biomarkers which are derived from complete blood count, predicting outcomes of neonatal sepsis is still challenging. Conventional screening tests such as total and differential leukocyte counts, band cells, neutrophil counts and rapid immunological tests like C-reactive protein (CRP) assays may help in the diagnosis of septicemias; however, they lack the capacity to predict the severity of sepsis (5).

One of the important aspects of acute infection is the decline in eosinophil count that spreads through the blood circulation quickly and persistently (6).Efforts have been made to diagnose EONS using an easy and cheap tools such as eosinophil count and neutrophil to lymphocyte ratio (NLR) (7).

From the previous studies, eosinopenia has a good sensitivity and specificity in diagnosing sepsis on both EONS and LONS, but they were using adult's sepsis cut off point (7).

Moreover, bacterial products and cytokines released during sepsis also delay neutrophil apoptosis,which contributes to the degree of sepsis severity (8).

In this study, we tried to use point of intersection from neonatal subjects. The diagnostic value of NLR in EONS alone has never been done in neonates in Egypt.

This study were purposed to provide information about diagnostic value of eosinophil count and NLR for detecting EONS.

Patients and Methods: The current study was a descriptive cross sectional study that conducted in the neonatal intensive care unit (NICU) in Fayoum University Hospital and Itsa Central Hospital from February to December 2019. Study subjects were all neonates who met the inclusion criteria. We used consecutive sampling method with a sample size of 100 neonates. Detailed history and meticulous general & systemic examinations were done, and also laboratory examination (hematology profile, differential count, eosinophil count, and blood culture).

Inclusion criteria:
1. Age (1-72 hours).
2. Any mode of delivery (vaginal or CS).
3. Both genders.
4. Sepsis clinical presentation (according to griffin score).
5. Sepsis risk factors: 2 major or 1 major and 2 or more minor

Exclusion criteria:
1-Any major congenital anomalies.
2-Any major surgical conditions.
3-Late onset neonatal sepsis (After 72 hours).
4- Healthy infants (term or preterm).

Statistical Methods
The collected data was organized, tabulated and statistically analyzed using SPSS software statistical computer package version 18 (SPSS Inc, USA).
□ **For quantitative data**:
The mean, standard deviation (SD) were calculated. Independent t-test was used as a test of significance.
□ **For qualitative data**:

The number and percent distribution was calculated and Chi-squared test was used as a test of significance.

☐ **For interpretation of** results of tests of significance, significance was adopted at P ≤ 0.05.

Ethical Consideration

The study was reviewed by the Faculty of Medicine Research Ethical Committee.The participants were informed by the researcher about the objectives of the study, the examination, investigations that were done. Also the confidentiality of their information was respected and their right not to participate in the study was ensured.

Results:

There were 100 neonates suspected with sepsis that fulfill the inclusion criteria as in figure 1.

Incidence of EONS were mostly found in males, 61 (61%), compared with females, 39 (39%).

Blood culture were made and resulting in 37 positive growth, consist of klebsiella (16), MRSA (7), E.coli (4), staphylococcus aureus (3), CONS (3), gram negative bacilli (3) and acinobacter (1) .

Mean eosinophil count from the EONS group was 250 ± 170 cells/mm3, meanwhile from non-EONS group was 670 ± 470 cells/mm3 with p<0.001 considered as significant. We also tallied their NLR and found that mean NLR from EONS and non-EONS group was 3.08 ± 1.9 and 1.29 ± 0.76, respectively, with p<0.001 considered as significant also. Figure 2A describes data distribution of eosinophil count in EONS and non-EONS group, while figure 4 describes data distribution of NLR in both groups.

An ROC curve to analyze the ability of eosinophil count to diagnose EONS (Fig.5) demonstrates that with a cut-off of 280 cells/mm3, there is a 56.3% sensitivity and a 93.1% specificity,also the positive predictive value (PPV) was (96.8%) and the negative predictive value (NPV) was (36.2%) .

A similar analysis with NLR showed that the best specificity and sensitivity for EONS was at NLR 1.75 with sensitivity of 70.4% and specificity 75.9%, also the positive predictive value (PPV) was (91.7%) and the negative predictive value (NPV) was(40.5%)

Discussion:

In our study, neonates suspected with sepsis were found more in males than females. There were **100** neonates, in whom 61 (61%) were males, 55% of cases were preterm with average gestational age 34.6 ± 3 weeks , average weight was 2.1 ± 0.7 kg , According to mode of delivery, 76 % of neonates suspected with sepsis were delivered by caesarean section, compared to 24 % of neonates that were delivered normally, 37% of cases were culture proven cases of neonatal sepsis.

These findings were in accordance with *Yogeeta et al* (9) , out of 150 clinically suspected neonates, 44 (29.3%) were culture proven cases of neonatal sepsis. The males (70.4%) were predominantly affected. Among neonates with EONS, 93.1% were low birth weight and 86.3% were preterm. According to mode of delivery, 52.2 % of neonates with sepsis were delivered by caesarean section, compared to 47.7 % of neonates that were delivered normally.

This study showed that sepsis risk factors for the selected cases,57% of mothers with premature rupture of membranes (PROM) >18 hours, 42% with history of intrapartum maternal fever, 3% with history of chorioamnionitis, 8% of cases were one of twins or triple, 34% of mothers were having urinary tract infection(UTI) and 41% with vaginal infections**.**

Yogeeta et al (9), Study of maternal and neonatal risk factors showed significant association with premature rupture of membranes, (p value=0.0001), maternal fever (p value=0.01) and birth asphyxia (p value=0.007) with neonatal sepsis by using multivariate analysis.

We should have at least 2 clinical abnormal presentations, so these were the clinical presentations of our cases (10).

About **91**% of cases presented with respiratory distress and need increase oxygen requirement, **89**% with poor suckling and **60**% with poor reflexes, **2**% with convulsions and **4**% were having (bradycardia ,apnea ,fever and hypothermia).

On the other hand *,Velaphi et al*,(11) reported in their study that the most common signs of sepsis included tachypnea (32%), metabolic acidosis (36%), lethargy (15%), and hypothermia (15%); whilst fever was uncommon (0.6%).

Laboratory investigations were done to all cases to confirm or exclude EONS in the form of :

> ➢ Complete blood picture with differential.
> ➢ I/T ratio.
> ➢ C-reactive protein.
> ➢ Blood culture.

If there were at least 2 laboratory changes (leukocytosis or leucopenia, thrombocytopenia , I/T ratio ≥0.2) with or without positive blood culture so the case was confirmed EONS (10).

Regarding blood culture in our study, **37%** of cases showed positive blood culture. This comes in agreement with *Ferrieri et al*,(12) study showing that positive blood culture only found in 30-40% sepsis cases.

Complete blood count was done to all cases and showed that 39% of cases were having thrombocytopenia (<100000), 52% of cases with leukopenia (<5000) or leukocytosis (>20000), and increased I/T ratio (≥0.2) in 22% of cases .

This was in agreement with, *Medhat et al*,(13) case control study that revealed: WBCs were statistically significantly($P = 0.030$) higher among cases compared to normal controls, as the vast majority of controls had normal WBCs count (96.7%), while only one case had leucocytosis (3.3%). Among cases leucycytosis was seen in (48%) and leucopenia was seen in (4%).Moreover, platelet count showed a statistically significant ($P < 0.001$) difference between cases and controls, with many of cases showing low platelet count. All of the controls had a normal PLT count, while 30% of cases had thrombocytopenia, the difference was statistically significant ($P = 0.001$).

Regarding other markers of diagnosing sepsis, CRP level was elevated in 85% of cases in this study .This finding was in agreement with *Sidra et al* (14) who found that the mean CRP level was significantly higher in patients with sepsis than controls.

Our cases were classified into 2 groups: confirmed sepsis (71%) and non-confirmed sepsis group (29%) ,to compare eosinophils and NLR between the 2 groups.

In this study, mean eosinophil count was 0.25 ± 0.17 k/microliter =250 ±170 cells/mm3 from the EONS group and as for non-confirmed EONS group it was 0.67 ± 0.47 k/microliter = 670 ± 470 cells/mm3 . Based on the logistic regression analysis, a very significant correlation was found between eosinophil count and sepsis occurrence with p value (**<0.0001 (S)**), also the positive predictive value (PPV) was 96.8% and the negative predictive value (NPV) was 36.2% .

The ROC analysis showed AUC 80.8% with an eosinophil cut-off point of 280 cells/mm3 and sensitivity = 56.3%, specificity = 93.1%.

Our results were in agreement with *Wilar,*(15) who reported that mean eosinophil count was 169.8 ± 197.1 cells/mm3 from the EONS group and as for non-EONS group it was 405.7 ±288.9 cells/mm3 . Based on the logistic regression analysis, a very significant correlation was found between eosinophil count and sepsis occurrence with (p<0.001), where the lower the eosinophil count is, the higher the chance of sepsis to take place.

Our results were in concordance with those obtained by *Bagus et al,* (16) who conducted a study to compare the diagnostic values of immature granulocytes, eosinopenia and IT ratios in detecting EONS in neonates (0-6 hours) with the risk of bacterial infection, where as eosinopenia showed the highest specificity of detection to detect EONS.

This study also looking for NLR corellation with EONS, and we found that mean NLR was 3.08±1.9 from the EONS group and 1.29± 0.76 for non-EONS group. Based on the logistic regression analysis, a very significant correlation also found between NLR and sepsis incidence, where the higher the ratio, the higher the incidence of EONS as well with p value (<0.0001), also the positive predictive value (PPV) was 91.7% and the negative predictive value (NPV) was 40.5% .

Based on the ROC curve with a 1.75 cut-off point, the result shows 70.4% sensitivity, 75.9% specificity.

Our results were in agreement with *Wilar*,(15) who found that the mean NLR from the EONS and the non EONS group was 2.82±2.29 and 0.82±0.32, respectively, also with (P<0.001) considered as significant.

A similar analysis with NLR showed that the best specificity and sensitivity for EONS was at NLR 1.245 with sensitivity of 83.3% and specificity 93.3%, and these results are near to our results.

On the other hand *Dursun et al*,(17) reported that NLR had a sensitivity of 75.6%, a specificity of 38.4%, a PPV of 35.6% , and a NPV of 77.8 % to predict sepsis in children.

*Yu et al,(*18) reported that multiple studies have supported the role of the neutrophil-to-lymphocyte ratio (NLR) as a prognostic tool, demonstrating worse outcomes with higher NLR values.

Limitations of this study is there was no follow-up examination in patients with higher levels of eosinophil counts and low NLR. To determine the risk of mortality in neonates with neonatal sepsis, serial examination of eosinophil and NLR is required.
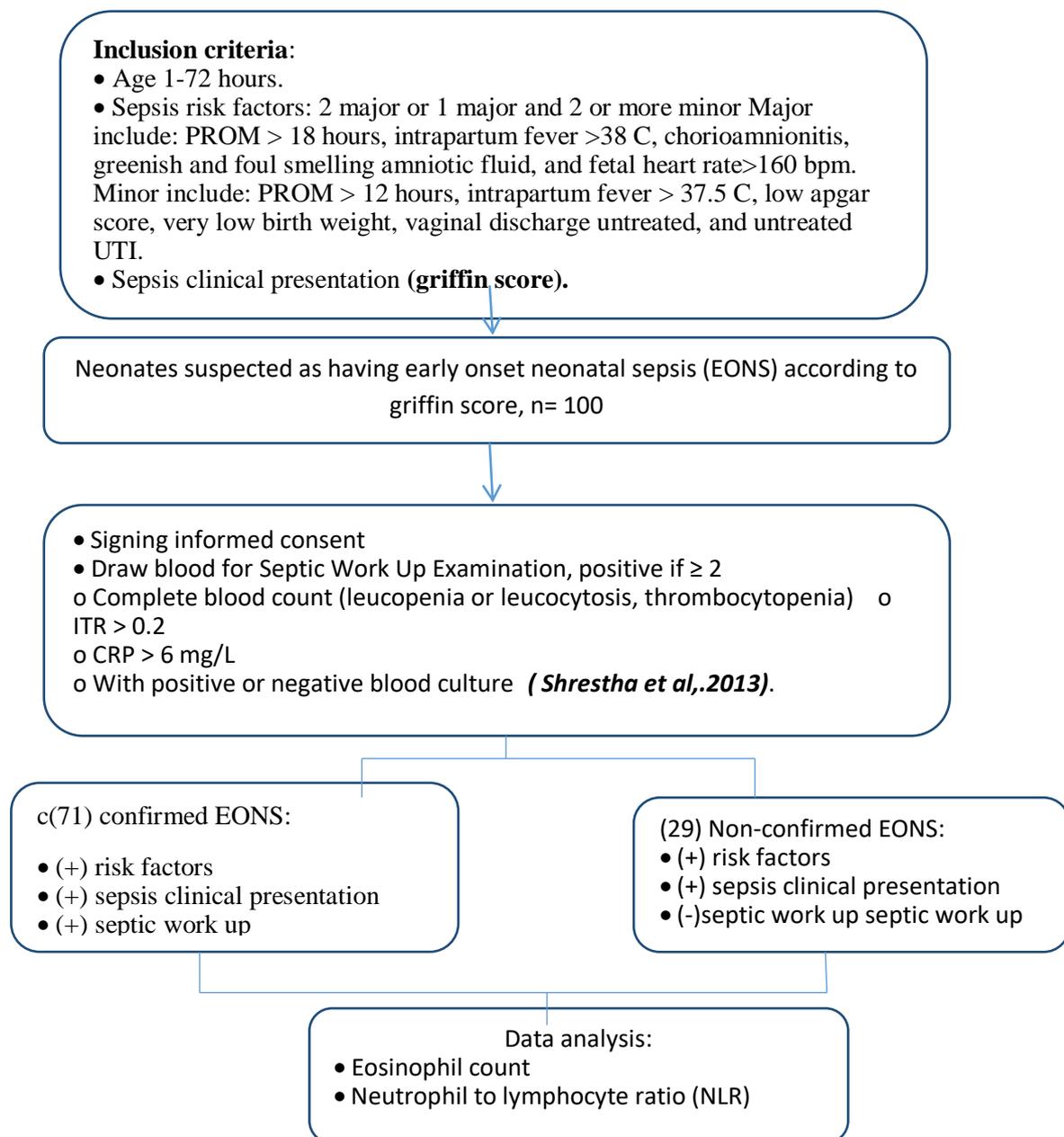
Conclusion:

From our study we concluded that eosinopenia and an increased in neutrophil to lymphocyte ratio (NLR) both have a high specificity value as a diagnostic marker of EONS.
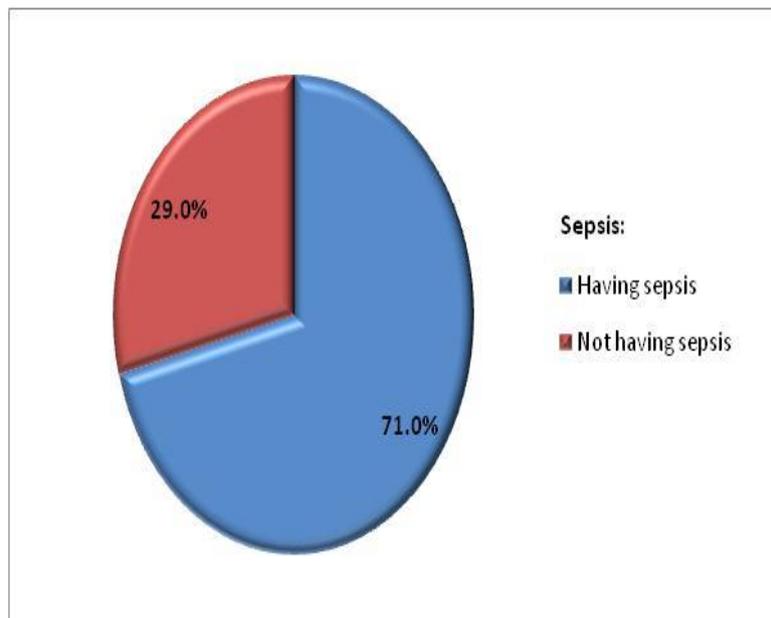
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Figure (1): Inclusion criteria:

Inclusion criteria:
- Age 1-72 hours.
- Sepsis risk factors: 2 major or 1 major and 2 or more minor Major include: PROM > 18 hours, intrapartum fever >38 C, chorioamnionitis, greenish and foul smelling amniotic fluid, and fetal heart rate>160 bpm. Minor include: PROM > 12 hours, intrapartum fever > 37.5 C, low apgar score, very low birth weight, vaginal discharge untreated, and untreated UTI.
- Sepsis clinical presentation **(griffin score).**

Neonates suspected as having early onset neonatal sepsis (EONS) according to griffin score, n= 100

- Signing informed consent
- Draw blood for Septic Work Up Examination, positive if ≥ 2
o Complete blood count (leucopenia or leucocytosis, thrombocytopenia)   o ITR > 0.2
o CRP > 6 mg/L
o With positive or negative blood culture *(Shrestha et al,.2013)*.

c(71) confirmed EONS:

- (+) risk factors
- (+) sepsis clinical presentation
- (+) septic work up

(29) Non-confirmed EONS:
- (+) risk factors
- (+) sepsis clinical presentation
- (-)septic work up septic work up

Data analysis:
- Eosinophil count
- Neutrophil to lymphocyte ratio (NLR)

Figure (2):Proportion of Confirmed
Sepsis and Non Confirmed Sepsis Groups

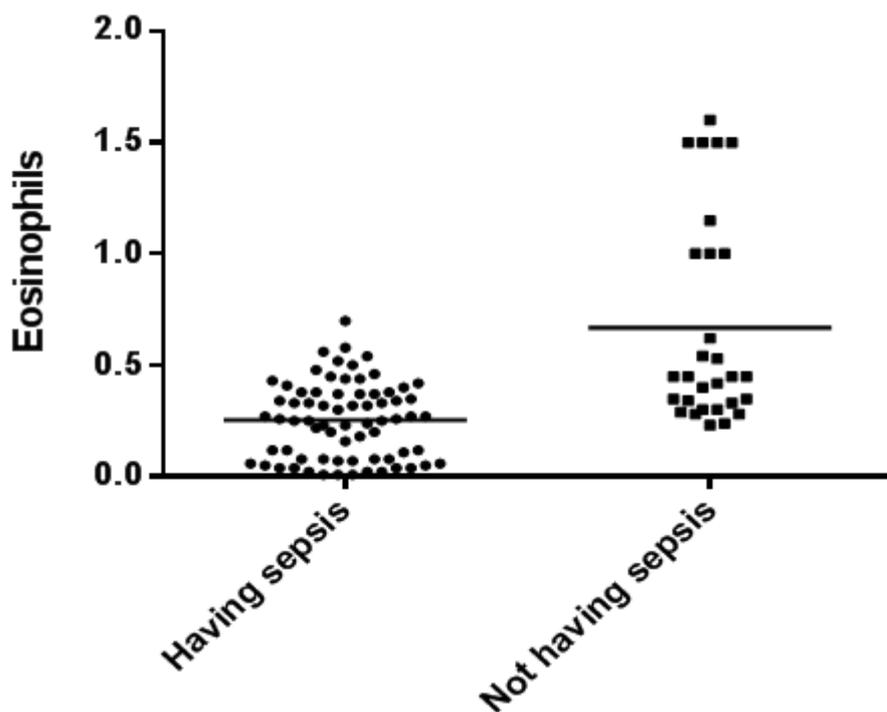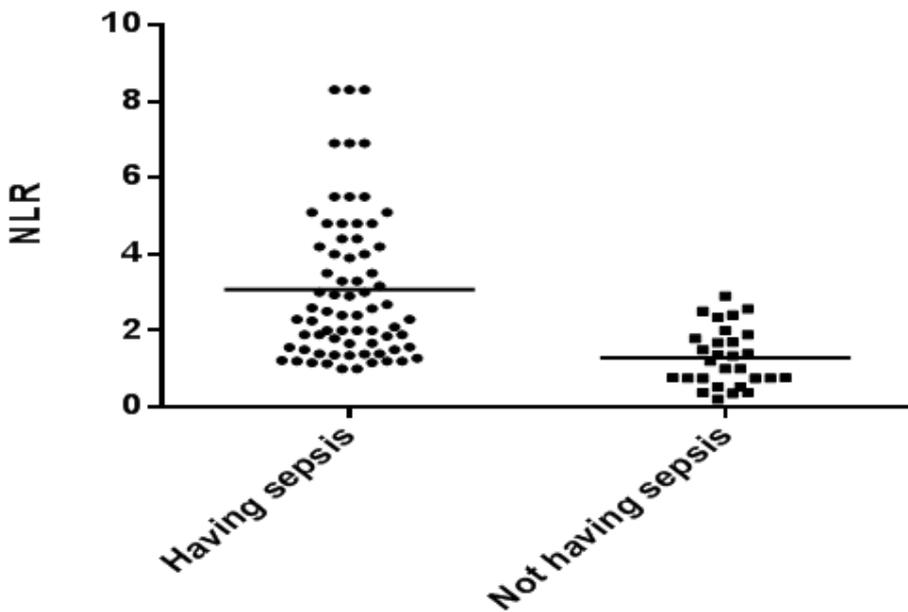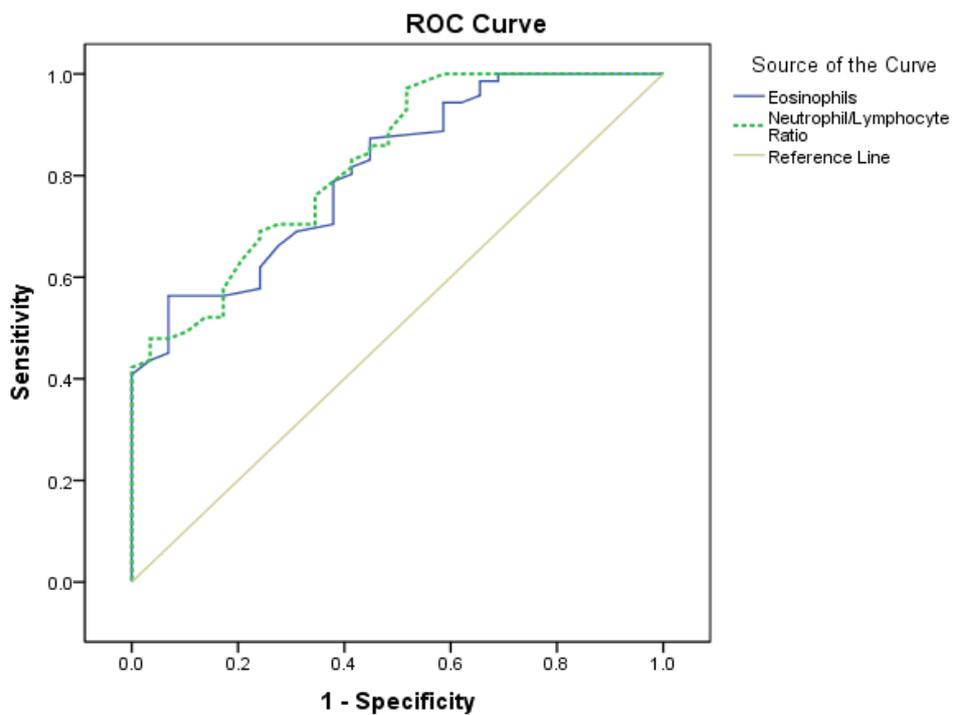**Figure (3): Proportion of Eosinophils Among the Study Groups**

Figure (4):Proportion of NLR Among the Study Groups

Figure (5):ROC Curve for Detecting EONS