



## Prognostic Significance of Leukocyte Differentials in COVID-19

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### Abstract

**Introduction:** The new coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has spread to many countries around the world, causing a global outbreak of COVID-19. The world is burdened by high morbidity and mortality caused by it. The complete blood count is the most available, efficient and economic examination. This study aims to analyse the leukocyte differentials of patients at the time of admission and the prognosis of COVID-19 in terms of mortality.

**Materials and methods:** Cross-sectional observational analysis of 123 patients who are COVID-19 positive admitted at Government Hospital for Chest and Communicable Diseases/ Andhra Medical College, Visakhapatnam between July 2020 to November 2020. Prognosis of patients assessed in terms of mortality. Statistical analysis was done using SPSS version 21. Cross validation of the model was done using R software.

**Results:** Of the 123 patients, 31.7% were in Category A, 21.7% in category B and 47.2% in Category C according to MOHFW India. 76% survived and 23.6% died during disease. Logistic regression to predict mortality showed an increase in WBC has p value of 0.0465 and an increase in neutrophil has p value of 0.05. Logistic regression to predict mortality based on NLR, LMR and PLR showed significant p value for NLR (0.0452) and PLR(0.0349). The model had a predictive accuracy of 64.8% with 33.3% false negatives.

**Conclusion:** Older age, leukocytosis, neutrophilia, lymphocytopenia, monocytopenia, high NLR and low PLR were indicative of severe COVID-19 disease.

**Keywords:** COVID-19, Leukocyte differentials, Prognosis

### INTRODUCTION

The COVID-19 disease caused by a novel coronavirus was first identified in December 2019 in Wuhan, China and spread rapidly throughout the world. The causative agent is an RNA beta coronavirus named severe acute respiratory syndrome coronavirus 2(SARS-CoV-2). It was declared to be a global pandemic on March 11, 2020.

COVID-19 has a wide range of clinical spectrum which varies from asymptomatic to critical infection [1]. The common clinical features of COVID-19 are

fever, dry cough, shortness of breath, myalgia, anosmia, ageusia and fatigue.

The understanding of the pathophysiology of COVID-19 has increased presently [2]. The virus is transmitted via respiratory droplets and aerosols from person to person. In about 80% of patients, containment of infection occurs with viral clearance in 10-14 days. In the remaining 20% of patients, invasion and infection of the type 2 pneumocytes occurs leading to the release of multiple

inflammatory mediators known as the cytokine storm. This will lead to the recruitment of neutrophils. This along with apoptosis of pneumocytes during viral replication led to diffuse alveolar damage eventually culminating in an acute respiratory distress syndrome (ARDS). Other common complications associated with COVID-19 disease are sepsis, disseminated intravascular coagulation, acute liver and kidney injury and pulmonary embolism. So, determining the predictive indicators of severe infection is of great importance.

A complete blood count is the most commonly performed haematological laboratory test worldwide. The present study focuses on whether we can predict the prognosis of COVID-19 from complete blood analysis.

In a study conducted on 148 hospitalized COVID-19 patients, higher total leukocyte count, neutrophilia, lymphopenia, and high NLR are associated with severe COVID-19 [3]. In another study on 93 hospitalized COVID-19 patients, NLR was found to be an independent biomarker for indicating poor clinical outcomes [4].

We evaluate the association between peripheral blood WBC count, differential WBC counts, neutrophil to lymphocyte ratio (NLR), lymphocyte-to-monocyte ratio (LMR) and platelet to lymphocyte ratio (PLR) at the time of hospital admission and the prognosis of COVID 19 in terms of mortality.

## MATERIALS AND METHODS

*Study design:* Cross sectional observational analytical study

*Study population:* 123 patients with lab confirmed COVID-19 admitted between July 2020 to November 2020 at Government Hospital for Chest and Communicable Diseases/ Andhra Medical College, Visakhapatnam.

*Inclusion criteria:*

1. Age > 18 years
2. Nasopharyngeal/ oropharyngeal swab for RT-PCR or TrueNat positive for COVID-19
3. Patients with complete blood count at the time of admission

*Exclusion criteria:*

1. Age < 18 years
2. Patients with haematological malignancies
3. Immunosuppressed patients
4. Patients on steroids before the initial blood test
5. Patients with documented secondary bacterial infections.

*Methodology:* The subjects were selected from the population of patients by simple random sampling. After excluding data with missing values, 123 patients were selected for study who met the above criteria. Patients were grouped into COVID-19 categories based on guidelines by Ministry of Health and Family Welfare (MOHFW), India [5] (Table 1). Clinical history of patients was noted, initial blood workup using standard methods and appropriate treatment as per the Government guidelines was provided. The prognosis of patients was assessed in terms of mortality. Statistical analysis was done using SPSS Version 21. Cross validation of the model was done using R software.

## RESULTS

The study included 123 patients of which, 84 (68.3%) were males and 39 (31.7%) were females. The predominant age group in the study was 46-55 years. 39 people (31.7%) were included in Category A, 26(21.7%) in Category B and 58 (47.2%) in Category C according to the guidelines by MOHFW India (Figure 1).

Out of 123 patients, 97 (78.9%) had fever, 80(65%) had cough, 71(57.7%) had shortness of breath 28(22.8%) had myalgia, 14(11.4%) had sore throat, 5(4.1%) had anosmia or aguesia, 27(21.9%) had other symptoms like vomiting, diarrhoea, headache, rhinitis, palpitation and chest pain (Figure 2). Majority of patients presented at around 7 days of symptom onset (Figure 3).

Out of the 123 patients, 41 (33.3%) had Diabetes Mellitus, 37(30.1%) had hypertension, 6(4.9%) had COPD, 5(4.1%) had tuberculosis, 3(2.4%) had malignancies, 19(15.4%) had other comorbidities like bronchial asthma, hypothyroidism, renal disease, coronary artery disease, cerebrovascular disease, seizure disorder, gall stones, kyphoscoliosis.

94 people (76.4%) survived COVID-19, whereas 29 people (23.6%) died during COVID-19. In the survivor group, 61(64.9%) were males and 33(35.1%) were females. In the non-survivor group, 23(79.3%) were males and 6(20.7%) were females.

COVID-19 mortality increased as age increased. Logistic regression to predict mortality with age had a significant p value of 0.050 (Table 2, Figure 4).

The mean WBC count among the survivor group was 8415 per mm<sup>3</sup> and non-survivor group was 10211.37 per mm<sup>3</sup>. The mean neutrophil count among survivor group was 6324 per mm<sup>3</sup> and non-survivor group was 8106.37 per mm<sup>3</sup> (Table 3). Independent t-test for means of WBC has p value of 0.0465 with the area under ROC curve 0.599 and an increase in neutrophil has p value of 0.05 with the area under ROC curve 0.615 (Figure 5).

Among the survivors, 44.8% had abnormal lymphocyte count. Out of which, 84.6% had lymphocytopenia and 15% had lymphocytosis. But compared to survivors, this was not statistically significant. Monocyte count in non-survivor group showed monocytopenia in 62% of patients and normal values in 32% of patients. But this is also not statistically significant when compared with non-survivor group.

The mean NLR of survivor group was 4.77 and non-survivor group was 5.62, mean LMR of survivor group was 7.29 and non-survivor group was 6.74 and mean PLR of survivor group was 204.73 and non-survivor group was 167.88 (Table 4).

Logistic regression model to predict mortality based on NLR, LMR and PLR showed significant p value for NLR (0.0452) and PLR (0.0349) (Table 5). The area under the ROC curve for this model is 0.69 (Figure 6). Cross validation for the accuracy of the model was done using all but one cross validation method. With the threshold probability of the model at 0.24, we performed a 70:30 split of sample data and trained the model using 70% of data and predicted mortality for the remaining 30%. We got an accuracy of 64.8% and false negatives of 33.3% (Figure 7).

## DISCUSSION

Mortality was observed to increase as age progresses, with the maximum in the 66 to 75 years of age group.

A similar observation was found in several other studies[3].

Increased total leukocyte count and differential neutrophil count was seen in the non-survivor group. There was lymphocytopenia and monocytopenia in the non-survivor group although it was not statistically significant.

We found a higher NLR ratio as a poor prognosis factor in COVID-19 patients. In a study by Liu Y *et al* on 245 COVID-19 patients, NLR was an independent risk factor of in-hospital mortality [6]. The neutrophil to lymphocyte ratio is elevated reflecting the systemic inflammation. Neutrophils are elevated in response to the cytokine storm. The triggered neutrophils will release Reactive Oxygen Species and cytotoxic mediators to dampen viral infection. Neutrophils also release neutrophil extracellular trap (NET), which is a sticky web of DNA conjugated with antimicrobial enzymes (MPO, histones) resulting in the capture and killing of different pathogens including viruses. Lymphocytes don't show a significant decrease in the early stage of viral infection. But it is significantly decreased in severe and critically ill patients. Lymphocytopenia occurs as they express ACE-2 receptors which are the target of the virus leading to direct damage of cells. Other reasons for lymphocytopenia are migration of lymphocytes from peripheral blood to lung, defective hematopoiesis and apoptosis of lymphocytes as a response to hyperinflammation. A meta-analysis including 4,911 COVID-19 patients from 29 studies found that surveillance of NLR may help clinicians identify high-risk COVID-19 patients at an early stage [7]. A meta-analysis including 6,320 patients from 52 eligible articles also pointed out a similar point [8].

PLR also indicate the degree of the cytokine storm. We got a low PLR ratio at the time of admission in the non-survivor group. This is similar to observation by Qu *et al* in a study where they noticed a low PLR in non-survivor group at hospital admission and an increase in platelet and PLR ratio during hospitalization reflecting hyperinflammation [9]. Platelets are immune cells with a role in innate immunity, inflammatory response, hemostasis, coagulation, vascular integrity maintenance, angiogenesis and tumor biology. Coronavirus directly invaded bone marrow stromal cells leading to

hematopoietic inhibition. Lungs may be one of the organs in which mature megakaryocytes release platelets and thrombocytopenia in patients with COVID-19 may be associated with lung damage. Lung tissue damage due to COVID-19 along with endothelial injury leads to activation, aggregation and retention of platelets in the lung and formation of thrombus at injured sites. Thus there is a depletion of platelets and megakaryocytes. During the treatment period, an increase in the release of cytokines occur and may lead to an increase in platelets.

The limitations of the present study are – small sample size, cross sectional study design, single facility study and the study is based on blood parameters at the time of admission alone.

## CONCLUSION

Older age, leukocytosis, neutrophilia, lymphocytopenia, monocytopenia, high NLR and low PLR were indicative of severe COVID-19 disease. Early recognition of severe cases of COVID-19 allows for early triaging and timely initiation of management. These markers are cost effective and easily accessible in all laboratories.

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**TABLES**

CATEGORY	Type of patients who are provided treatment and care
Group A	Asymptomatic/ patients with mild symptoms RR<24/min & SpO2>94% at room air
Group B	Symptomatic patient with mild to moderate pneumonia with no signs of severe disease RR: 24-30/min (Or) SpO2: 90-94% at room air
Group C	Symptomatic patient with severe pneumonia with RR>30/ min (Or) SpO2<90% at room air (Or) less than 94% with oxygen, ARDS, Septic shock.

**Table 1: Clinical severity categories of COVID-19 according to MOHFW India**

	S.E.	Sig.
Age	.017	.050
Gender	.516	.161
Duration of symptoms	.067	.523

**Table 2: Logistic regression to predict mortality with age had a significant p value of 0.050. COVID-19 mortality increased as age increased. Gender and duration of symptoms at the time of admission were not associated with mortality.**

	Total patients (n=123)	Survivors (n=94)	Non Survivors (n=29)	P value
<b>WBC</b>	8839.1 (2700-24700)	8415 (2700-24700)	10211.37 (3700-20200)	<b>0.0465</b>
<b>Neutrophils</b>	6744.8 (1368-20254)	6324 (1368-20254)	8106.37 (2660-17952)	<b>0.05</b>
Lymphocyte	1680 (350-6000)	1689 (350-6000)	1652.44 (650-4970)	0.76
Monocytes	339.65 (40-1435)	349.71 (40-1435)	307.06 (50-781)	0.172
Eosinophils	268.02(9-1062)	260(9-1062)	283.41(62-995)	0.82
Hemoglobin	12.8(6.9-16.6)	13.26(6.9-16.6)	11.69(7.3-14.6)	0.38
Platelet	263715 (44000-8,43,000)	2,77,000 (78000-8,43,000)	2,20,655 (44,000-5,36,000)	0.22

**Table 3: Independent t test for comparing mean values of haematological parameters in survivor and non-survivor groups.**

	Total patients (n=123)	Survivors (n=94)	Non survivors (n=29)
NLR	4.97 (0.75-27.2)	<b>4.77</b> (0.75-27.25)	<b>5.62</b> (1.71-18)
LMR	7.16 (0.67-30)	<b>7.29</b> (0.67-30)	<b>6.74</b> (2.32-23)
PLR	196.07 (28.17-931.25)	<b>204.73</b> (32.59-931.25)	<b>167.88</b> (28.17-402.53)

**Table 4: Mean NLR, LMR and PLR values in survivor and non survivor groups**

COEFFICIENTS	ESTIMATE	STD. ERROR	Z VALUE	P VALUE
NLR	0.59169	0.29546	2.003	0.0452
LMR	-0.09393	0.23792	-0.395	0.6930
PLR	-0.79942	0.37896	-2.109	0.0349

Table 5: Logistic regression model to predict mortality based on NLR, LMR and PLR.

**FIGURES**

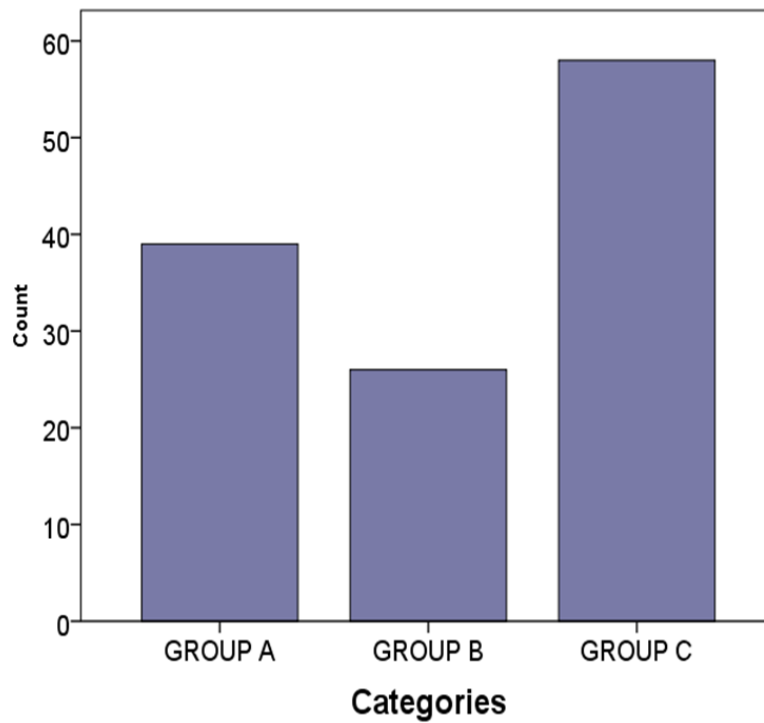


Figure 1: Grouping of patients into COVID-19 categories based on guidelines by MOHFW India

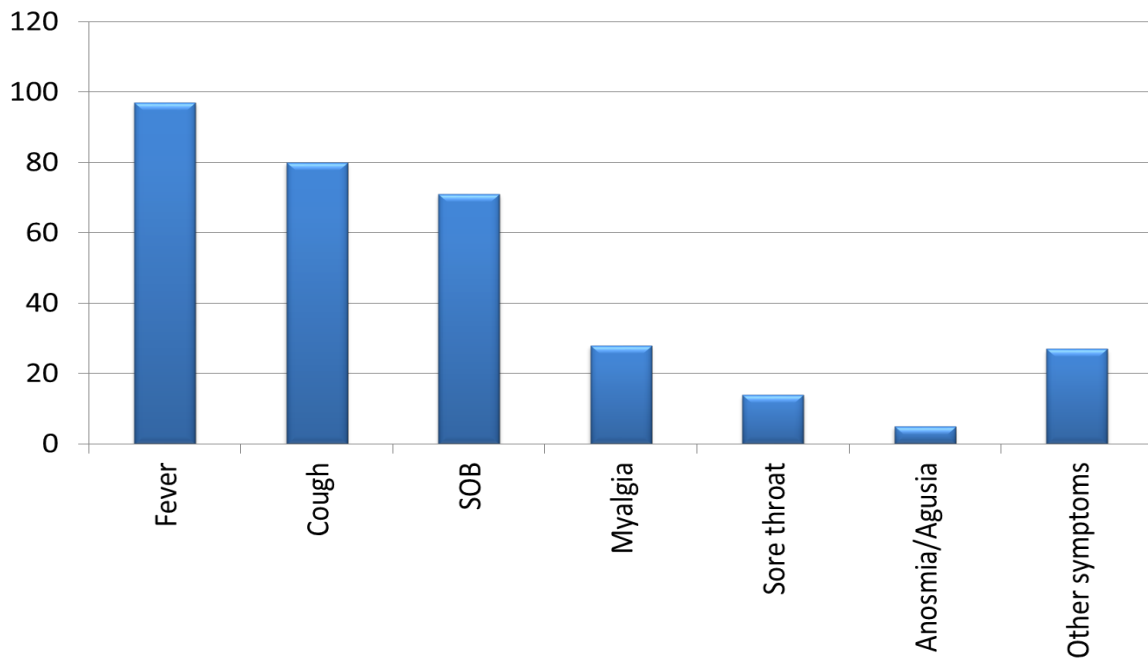


Figure 2: Symptoms of COVID-19 patients at the time of hospital admission

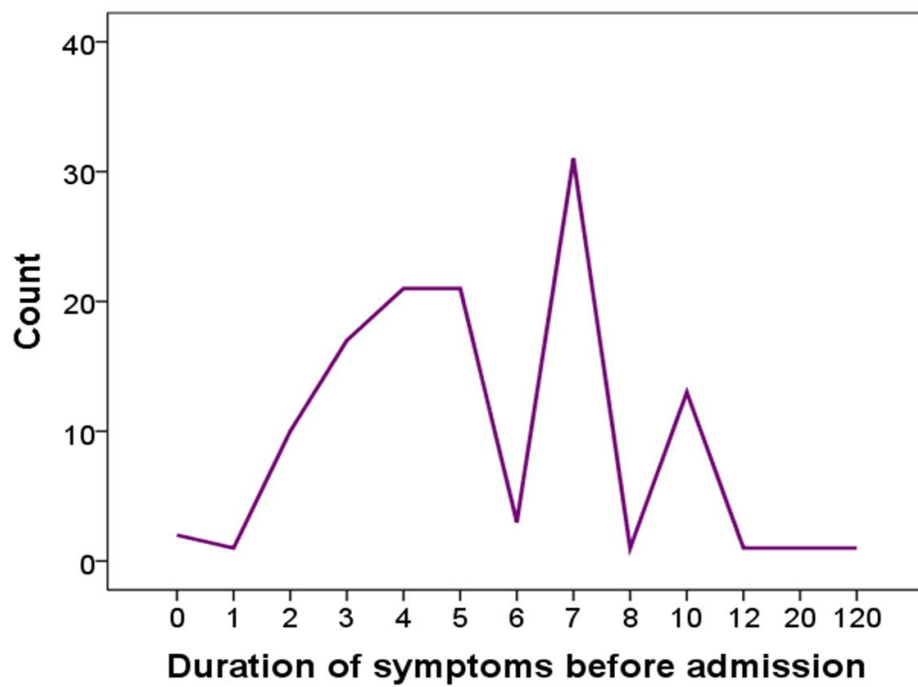


Figure 3: Duration of symptoms of COVID-19 patients at the time of hospital admission



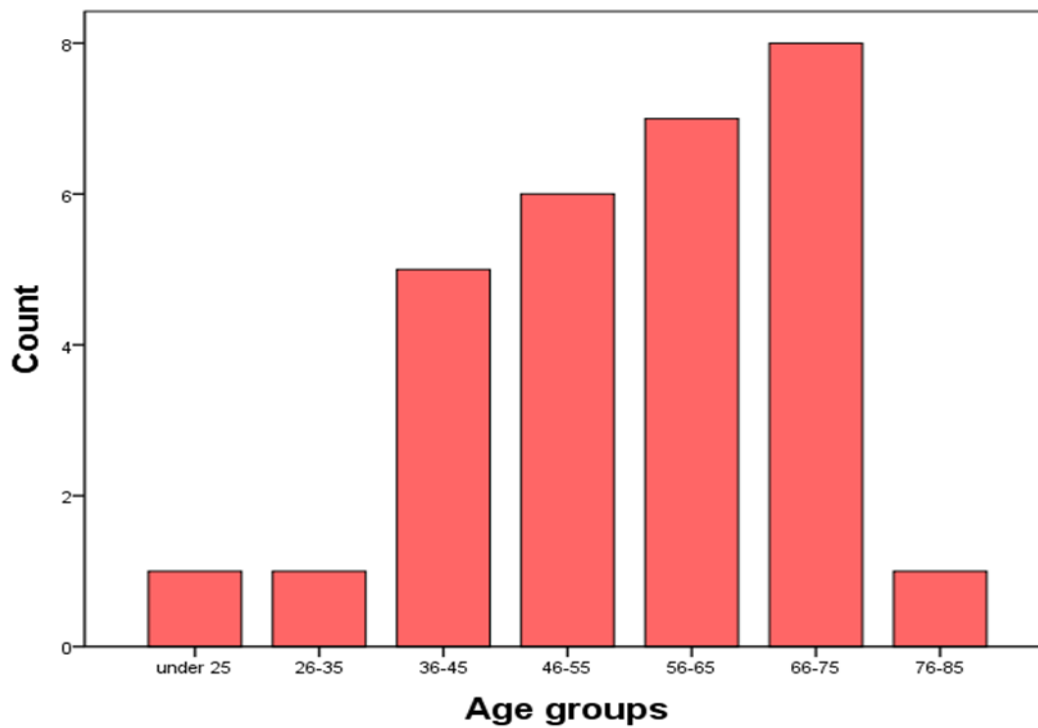


Figure 4: Age distribution among non survivors. Mortality increased as age increased.

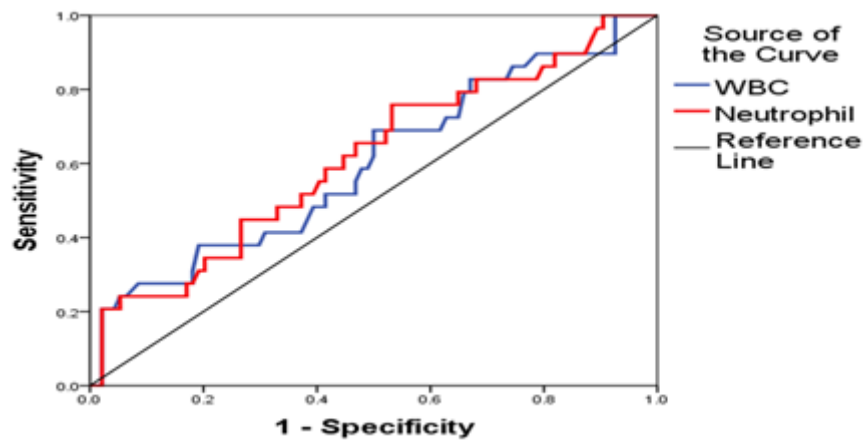
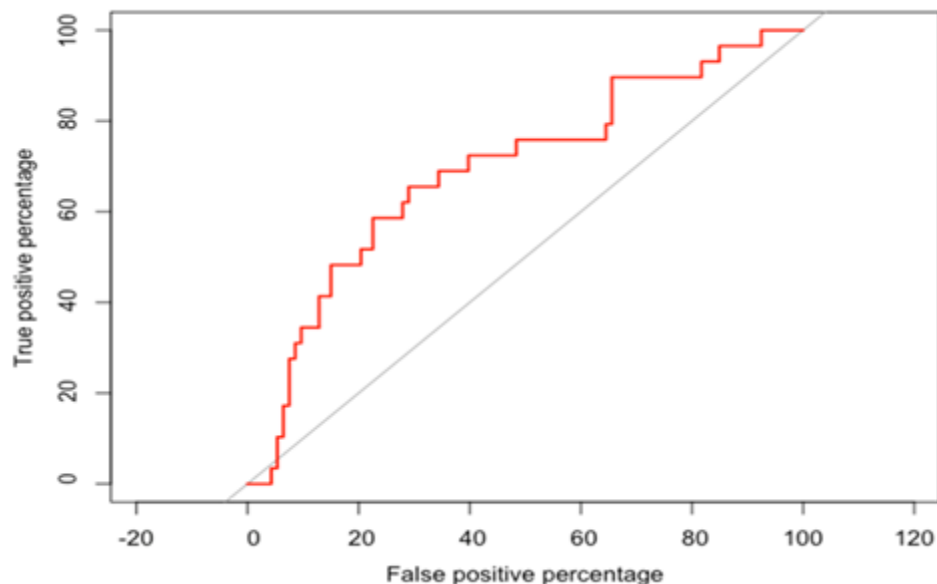
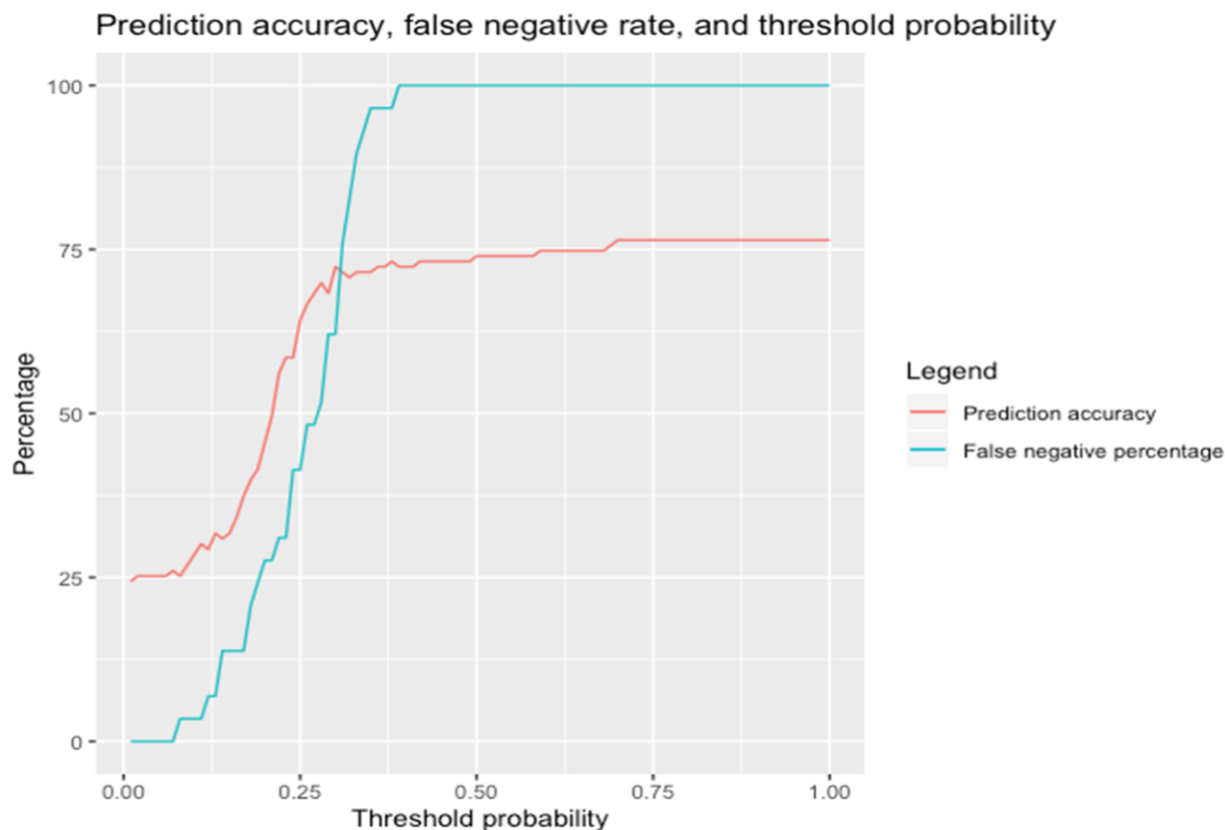


Figure 5: ROC curve for total WBC count and neutrophil count in prognosis of COVID-19 in terms of mortality. The area under the curve for WBC count is 0.599 and neutrophil count is 0.615



**Figure 6: ROC curve for the model involving NLR, LMR and PLR values to predict mortality in COVID-19 patients. The area under the curve is 0.69**



**Figure 7: Cross validation for prediction accuracy of the model was done using all but one cross validation method. With the threshold probability of the model at 0.24, we performed a 70:30 split of sample data and trained the model using 70% of data and predicted mortality for the remaining 30%. We got an accuracy of 64.8% and false negatives of 33.3%.**