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St. Paul's Hospital Millennium Medical College

Neutrophil-to-Lymphocyte Ratio as Predictor of Severity and Mortality in COVID-19 Patients in Millennium COVID-19 Care Center, Addis Ababa, Ethiopia.

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Addis Ababa, Ethiopia



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Abstract

Background: The global COVID-19 pandemic has infected millions of the world's population and resulted in huge stress on the health care system of all countries in the world. Early identification of patients who are at high risk of poor clinical outcomes is a key to success in the management of the disease and saving the lives of patients with coronavirus disease 2019 (COVID-19) in the context of limited medical resources. Neutrophil to Lymphocyte ratio is an easily available and cheap surrogate inflammatory marker, whether baseline NLR could be an independent predictor of in-hospital death and severity in the context of African COVID-19 patients remains to be investigated and standardized threshold level of NLR have not been well demonstrated.

Objective: This study aims to evaluate the role of NLR as a valuable predictor of Severity and in-hospital mortality in patients with COVID-19.

Methods: A cross-sectional study was conducted on patients with COVID-19 admitted to Millennium COVID 19 care center from August 1 to October 30/2021. The data was collected retrospectively and analysis was made on SPSS 26. Receiver Operating Characteristic curve analysis was used to calculate the area under the curve to assess the predictive capacity of NLR on mortality and severity, and to set the cutoff value. Binary and multivariable logistic regression analysis was performed to identify the association between independent variables and disease outcomes with an adjusted odds ratio (AOR), P-value, and 95%CI for AOR were used for testing significance.

Result: The NLR of 9.47 was identified as optimal cut-off value of predicting mortality with a sensitivity of 88.7% and a specificity of 95.4% (area under the curve (AUC): 0.95, 95% CI 0.92-98; $P < 0.0001$) and NLR of 5.86 was an effective threshold value in predicting the severity of disease with a sensitivity of 92.2% and specificity of 75% (AUC: 0.858, 95% CI 0.95-90; $P < 0.0001$). In multivariate logistic regression analysis, after adjusting for confounding factors, NLR more than 9.47 was significantly associated with all-cause of in-hospital mortality (AOR= 4.73, 95%CI, 1.19-33.68; $P < 0.02$), and value more than 5.86 was significantly associated with severity of disease (AOR=12.98, 95%CI 3.85-43.80; $P=0.001$).

Conclusion and recommendation

NLR is a simple inflammatory biomarker that is easily detected at admission, patients having $NLR \geq 9.47$ and ≥ 5.86 effectively predict in the hospital all-cause of mortality and severity of the disease. It provides an objective input for early decision-making inpatient management and allocation of resources.

Keyword: COVID 19, Neutrophil-to-Lymphocyte ratio

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Acronyms and Abbreviations

ACE2	Angiotensin-Converting Enzyme 2
ARDS	Acute Respiratory Distress Syndrome
ALC	Absolute Lymphocyte Count
ANC	Absolute Neutrophil Count
COVID 19	Corona Virus Diseases 19
CKD	Chronic Kidney Disease
COPD	Chronic Obstructive Pulmonary Disease
HIV	Human Immunodeficiency Virus
IFNs	Interferons
MERS	Middle East Respiratory Syndrome
MERSCOV	Middle East Respiratory Syndrome Coronavirus
MCCC	Millennium COVID-19 Care Center
NFκB	Nuclear Factor Kappa B
NLR	Neutrophil to Lymphocyte Ratio
NPV	Negative Predictive Value
PPV	Positive Predictive Value
PRR	Pattern recognition receptor
SARS COV2	Severe Acute Respiratory Syndrome Coronavirus 2
SOFA	Sequential Organ Failure Assessment Score
SPHMMC	Saint Paul Hospital Millennium Medical College
TMSP2	Trans Membrane Serine Protease 2
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
VOC	Variant of Concern
WHO	World Health Organization

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1. Introduction

1.1. Background of the study

Coronavirus disease 2019 (COVID-19), a highly infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has been rapidly spreading all over the world (affecting 223 countries) and remains a great threat to global public health (1).

The new coronavirus identified as the cause of the acute respiratory disease in humans since the end of December 2019 (2019-novel CoV) in Wuhan City, the capital of Hubei province in China after being isolated in the bronchoalveolar lavage fluid of three COVID-19 patients and later labeled as SARS-CoV2 by World Health Organization (2,3).

COVID -19 has been recognized as a global public health emergency on 31 January 2020 after cases had started to be seen outside china in less than two month period and subsequently, declared as a pandemic on March 11, 2020 (3).

According to the WHO COVID 19 dashboard, as of 26 May 2021, over 167,423,479 confirmed cases worldwide with a death toll of over a 3.4million. In Africa, more than 3,457,590 people are infected; the global case fatality rate of COVID-19 is approximately 3% (4).

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a member of the coronavirus family, a class of enveloped viruses with a positive-sense single-stranded RNA genome (5).

Genome-wide phylogenetic analysis indicates that SARS-CoV-2 is a genus Beta coronavirus and shares 79.5% and 50% sequence identity to SARS-CoV and MERS-CoV, respectively (5,6).

SARS-CoV-2 undergoes thousands of mutations since the start of the pandemic, more recently, there are four internationally confirmed variants of concern (VOCs) have been identified that appear to increase transmissibility, and potentially have an impact on disease severity and labeled as Alpha, Beta, Gamma, Delta and Omicron (7,8).

In addition, mutations can increase the virus' ability to evade adaptive immune responses from past SARS-CoV-2 infection or vaccination. This may lead to an increased risk of reinfection or decreased efficacy of vaccines (8,9).

SARS-CoV-2 use angiotensin-converting enzyme 2 (ACE2) and trans-membrane serine protease 2 (TMPRSS2) for cell entry, after virus entrance, intracellular antiviral mechanisms lead to the secretion of interferons (IFNs), which trigger an inflammatory response that characterized by an initial cytokine storm (hyper-inflammation) which result in acute respiratory distress syndrome (ARDS) followed by immune deregulation that causes sepsis-related fatalities like septic shock, thromboembolism, and multi-organ failure (10–12).

The main route of transmission is through respiratory droplets by direct contact with infected persons or by contact with contaminated objects and surfaces. The typical incubation period of COVID-19 ranges from 1 to 14 days, on average of 5–6 days (12,13).

The most common symptoms are fever, cough, myalgia, and fatigue; less common symptoms include sputum production, headache, hemoptysis, and diarrhea. More than half of patients developed dyspnea (14).

Most people with COVID-19 develop only mild (40%) or moderate (40%) disease, approximately 15% develop a severe disease that requires oxygen support, and 5% have a critical disease with complications. Several factors have been identified as risk factors for the disease severity and death including comorbidities (i.e. diabetes, hypertension, obesity, chronic lung disease, cancer, and cardiovascular diseases), age >65, current smoking, ethnicity, and genetic predisposition (12,15,16).

In terms of laboratory tests, the main characteristics of COVID-19 are normal or decreased a total number of white blood cells, decreased lymphocyte count, increased IL-6, CRP, ESR, procalcitonin, liver enzymes, D-dimer, ferritin, and lactate dehydrogenase (9,12).

Some of these laboratory markers (D-dimer, ferritin, IL-6, and deranged cell count) are implicated as an indicator of disease severity, progression, and outcome, As a new inflammatory indicator, neutrophil to lymphocyte ratio (NLR) changes can not only reflect the role of neutrophils in infection but also reflect the changes of lymphocytes. It has been reported that NLR has some predictive value in the diagnosis of disease, severity, and mortality in patients with COVID-19 that may potentially aid in risk stratification models for predicting severe and fatal COVID-19 (9,12,17,18).

1.2. Statement of the problem

The Global COVID-19 pandemic is the worst health crisis that the earth planet has faced in the past 75 years, it has infected millions of the world's population at a shocking speed and resulted in huge stress on the health care system of all countries in the world, even very robust health systems can be rapidly overwhelmed (19).

The impact of the pandemic is not only on the health system but also social and economic; the global economy envisages a 5.2% contraction in the gross domestic product (GDP) and stalls the progress made to achieve the Sustainable Development Goals (20–22). It will drag African economies into a fall of about 1.4% in GDP, with smaller economies facing a contraction of up to 7.8%, and similarly, the Ethiopian economy will face a \$1.3 billion (2.2%) GDP loss at the low base scenario (23).

The crude mortality rate varies substantially by country depending on the populations affected. The global clinical case fatality is currently over 3%, increasing with age and rising to approximately 15% or higher in patients over 80 years of age (4,19).

The fast spread of the disease raises concerns over the need for intensive care which is seen in one of the previous reports that mention one in 20 patients required intensive care (24). That may overwhelm health care system resources, especially in developing countries like Ethiopia where a significant shortage of ICU setup, limited manpower, and resources (17,22,24). Till now there are different biomarkers evaluated to identify the predictor potential of mortality and severity of the disease, some of these biomarkers (D-dimer, IL-6, lactate dehydrogenase, prothrombin time, and activated partial thromboplastin time) are not routinely and easily measured, hence, investigating simple reliable and accurate biomarker is mandatory, especially for resource-poor countries (17,18).

Neutrophil to lymphocyte ratio (NLR) is one of readily available cheap biomarker, which currently evaluated its role in predicting severity and mortality in several studies, however, its use in the clinical setting has been overlooked due to its variability of cutoff point and study result in a different region of the world, and its significance remains unclear among COVID 19 patient in our locality and Africa at large.

Previous studies on this area only focused on the predictive potential of NLR of severity and mortality of the disease and use randomly extrapolated cutoff points from different studies done in the other area which determines variable cutoff values that may not be enough to apply in our population, Hence it is failing to use a specific cutoff value in our clinical practice because of lack of well-standardized cutoff value for our specific population.

Therefore, this study will evaluate the predictive values of NLR on severity and mortality in patients with COVID-19 admitted in millennium COVID-19 care center in Addis Ababa, Ethiopia, and will identify the standardized cutoff value of NLR on admission.

1.3. Significance of the study

As the pandemic of coronavirus disease 2019 (COVID-19) continues, prognostic markers are now being identified. The neutrophil-to-lymphocyte ratio (NLR) is an easily accessible value that has been known to correlate with inflammation and prognosis in COVID 19. Knowing and understanding NLR as a predictor of severity and in-hospital mortality by the clinician is crucial for screening, early referral of a case to an appropriate treatment center, providing evidence-based clinical management decisions, and prevention of fatal complications.

This biomarker with the other associated variables helps to describe the natural history of illness and clinical course of the disease which helps to inform patients and their families about the risk of death.

The study will also give a direction for policymakers and the epidemic response team for timely and fair allocation of resources, distribution of manpower, and development of admission guidelines to treatment centers based on the identified predictor. Furthermore, the result of this study will be helpful for researchers as baseline data on the study area and input for other prospective studies or a clinical trial.

2. Literature Review

2.1. Role of NLR as Predictor of Mortality

A retrospective cohort of 245 patients with COVID-19 admitted to the Zhongnan Hospital of Wuhan University, showed that higher NLR was significantly associated with an increased risk of all-cause death during hospitalization and 8% higher risk of in-hospital mortality for each unit increase in NLR, and found that NLR is an independent risk factor of the in-hospital mortality for COVID-19 patients, especially for male (25).

Similarly, a study on the same area was conducted in 131 patients, The NLR was significantly higher in non-survivors when compared to survivors and there were no significant differences in gender and comorbidities, but Compared to the survivors, the non-survivors were older (median age 80) and present with the initial symptom of dyspnea (26).

An observational retrospective study performed in Madrid, Spain, were included 119 patients with COVID-19, significant worse outcomes were seen in patients with higher NLR values at admission and older patient, and higher increasing speed of NLR was observed compared with follow-up patients (27).

The review of a prospective registry from two large urban safety-net hospitals in Louisiana shows revealed that NLR on day 2 is an independent predictor of in-hospital mortality. Similarly, on hospital day 5, NLR was found to be a strong prognostic factor for increased (28).

In Iran, retrospectively estimate one-month mortality since admission, shows that NLR as predictors of one-month mortality in COVID-19 patients, increased age, and WBC count was a great confounder (29).

Similarly, in a Double-Centre study of 1320 patients, in Tehran, Iran, the ratio shows that significantly higher in the death group as compared to the ICU group (29).` However, a retrospective cross-sectional study was conducted in 100 patients in the Chigateri General Hospital, in India, where NLR was not found to be significantly associated with mortality (30).

2.2. Role of NLR as a predictor of severity

A large multi-center retrospective study was enrolled 635 patients with COVID in 43 hospitals from ten provinces of China, showed revealed that increased NLR was associated with severe cases of COVID-19 (31).

A case-control study in three separate hospitals in Saudi Arabia were enrolled 701 confirmed COVID-19 patients (41 were ICU-admitted and 250 control subjects) showed that NLR was almost twice as of the non-intensive patients. However, the control group was tested negative for COVID-19 infection (32).

The prospective study was done on 61 patients with COVID-19 in Beijing Ditan Hospital, which showed that the neutrophil-to-lymphocyte ratio (NLR) was higher in the severe or critical group on admission and increased more rapidly compared to those in the mild or moderate group (33).

Another prospective cohort study of 352 hospitalized COVID-19 patients was done in Hub Province, which revealed that the NLR was significantly higher in patients with deterioration and serious clinical conditions like shock than in those without serious clinical outcomes (34).

2.3. Cut-off value of NLR for mortality and severity

A descriptive cross-sectional study was conducted in Pakistani, shows that $NLR > 3.13$ were predicted to develop a critical illness with age more than 50 years, however, due to the small sample size, the nature of the study and Confounding factors not well assessed and not powered enough (35).

Similarly, the prospective study was done in Beijing Ditan Hospital; $NLR \geq 3.13$ was identified as the cutoff value to predict the development of critical illness (33). Another multicenter study was done in china a cutoff value $NLR \geq 4.06$ and lymphocytes ≤ 0.765 were identified as predicting indicators for severe COVID-19 (30).

The review of a prospective registry from two large urban safety-net hospitals in Louisiana shows that $NLR > 4.94$ on day 1 predicted intubation ($P = 0.02$) and NLR above the established cutoff values on hospital days 2 and 5 each significantly predicted mortality ($P < 0.001$ and 0.002 , respectively) (28).

In Tehran, Iran the cutoff value of NLR was greater than 6.5 which may reflect the progression of the disease towards an unfavorable clinical outcome (29).

2.4. NLR as a risk factor

Another two stages retrospective cohort was done in China, the first single-center retrospective cohort study (between patients with COVID-19 and patients with non-COVID-19) found that higher NLR was an independent risk factor with the incidence of COVID-19.(31)

2.5. Other associated risk factors

Based on the South Korea nationwide data on 7590 patients with COVID-19, showed that hypertension, diabetes mellitus, congestive heart failure, chronic pulmonary disease, liver disease, renal disease, and cancer were significant risk factors for mortality after age and sex adjustment (16).

Similarly, a retrospective study was done on Tongji Hospital, Wuhan, China, Older age and underlying hypertension were identified as potential risk factors for severe COVID-19 and survival analysis revealed that male sex, older age, cardiac injury were associated with death in patients with severe COVID-19 (36).

A single-center case-cohort study of the 179 consecutive patients with confirmed and probable COVID-19 pneumonia was hospitalized to Wuhan Pulmonary Hospital, showed that age ≥ 65 years, preexisting concurrent cardiovascular or cerebrovascular disease were identified as predictors for mortality of COVID-19 pneumonia patients (37).

A multicenter cohort study was conducted in 65 hospitals across the US, revealed that older age (>80 years old), male sex, higher body mass index, active cancer, coronary artery disease, liver and kidney dysfunction were identified as an independent factor associated with COVID 19 related death (38).

Another case-control study done at Millennium COVID-19 Care Center (MCCC), Ethiopia, revealed that Having diabetes mellitus was found to be a significant predictor of death in severe COVID-19 patients (39). Similarly, A study done in Mekelle, Ethiopia COVID-19 treatment center on clinical spectrum and factors associated with risk of severe COVID-19 and in-hospital

mortality identify older age, diabetes, cardiovascular disease, malignancy, surgery/trauma, and human immunodeficiency virus infection as a factor associated with severe COVID-19 and older age, surgery/trauma, and malignancy as factors associated with risk of in-hospital mortality (40).

In the RECOVERY trial, treatment with dexamethasone conferred a survival benefit among participants who required supplemental oxygen at enrollment. In patients hospitalized with Covid-19, the use of dexamethasone resulted in lower 28-day mortality among those who were receiving either invasive mechanical ventilation or oxygen alone at randomization (41).

The Medical charts review was carried out in Tongji Hospital, Wuhan, highlighting the importance of early oxygen therapy after the onset of hypoxia symptoms and the risk of death increased significantly when oxygen therapy was started more than 2 days after hypoxia symptoms onset among critical patients (42).

A Retrospective, multi-center, national cohort study done in 16 intensive care units (ICU) in Spain, revealed that the long-term survival of mechanically ventilated patients with severe COVID-19 reaches more than 50% and may help to provide individualized risk stratification and potential treatment (43).

Systematic review and meta-analysis on Variation in the outcome of invasive mechanical ventilation between different countries for patients with severe COVID-19 showed that Mortality from invasive mechanical ventilation was highly variable among the included studies ranging between 21% and 100% (44).

A single-center prospective observational study was carried out in Medstar Georgetown University Hospital, showed that acute kidney injury requiring renal replacement therapy was associated with a high mortality rate similar to that associated with the need for mechanical ventilation, while multi-organ failure necessitating both techniques implied an extremely high mortality risk (45).

A multicenter cohort study at 67 hospitals across the United States revealed that AKI-Renal replacement therapy is common among critically ill patients with COVID-19 and is associated with a hospital mortality rate of 60%. Among those who survive to discharge, one in three still depends on RRT at discharge, and one in six remains RRTdependent 60 days after ICU admission (46).

2.6. Conceptual framework

The conceptual framework developed from a previous study conducted on COVID 19 severity and mortality associated with NLR, other clinical factors (socio-demography and comorbidity), laboratory and interventional measures in different times and places. The framework contains independent and dependent variables.

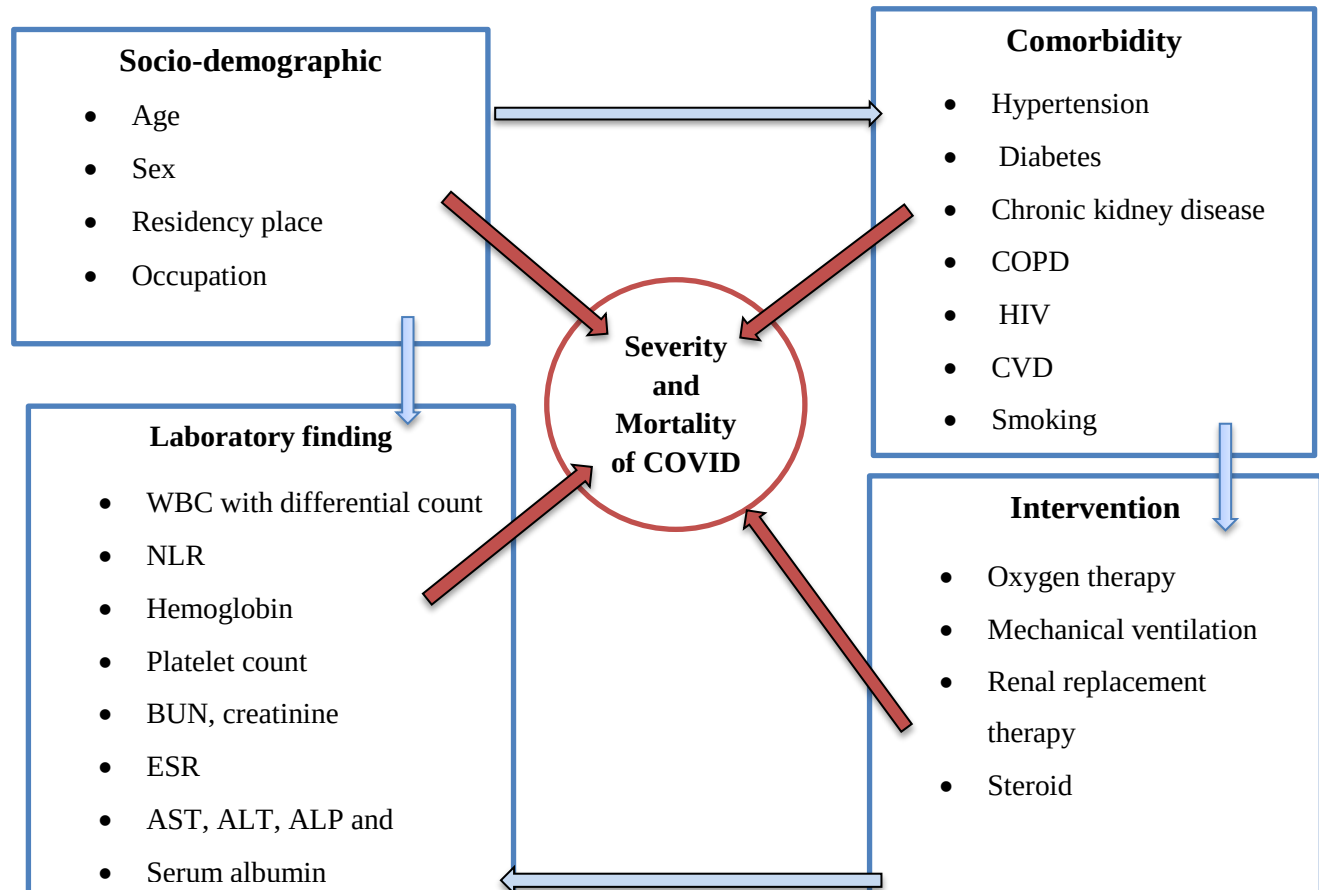


Figure1: Conceptual framework on COVID 19 severity and mortality associated with NLR and other clinical factors (socio-demography, comorbidity, and intervention role).

3. Objectives

3.1. General objective

To evaluate the role of NLR as a predictor of severity and in-hospital mortality in COVID 19 patients admitted in Millennium COVID-19 Care Center, Addis Ababa, Ethiopia, 2021

3.2. Specific objectives

- To determine whether the NLR can serve as a predictor of Severity among COVID 19 patients admitted in Millennium COVID-19 Care Center, Addis Ababa, Ethiopia, 2021
- To determine NLR as a predictor of in-hospital mortality among COVID 19 patients admitted in Millennium COVID-19 Care Center, Addis Ababa, Ethiopia, 2021.
- To establish the cutoff value of NLR that predicts the severity and mortality of COVID-19 patients admitted in Millennium COVID-19 Care Center, Addis Ababa, Ethiopia, 2021

4. Methods and Materials

4.1. Study Setting

The study was conducted in Millennium COVID-19 Care Center (MCCC), a makeshift hospital in Addis Ababa, located near Bole airport, is designed to accommodate 1040 patents, out of which 40 are intensive care unit beds. It started to receive patients on 02 June 20. The data was collected from January 24- February 7/2022 G.C

4.2. Study design

A Cross-sectional study was conducted in patients diagnosed with COVID-19 and admitted at Millennium COVID-19 Care Center (MCCC) and we compared the neutrophil to lymphocyte ratio (NLR) of survivors with non-survivors and sever with a non-sever form of COVID-19.

4.3. Source Population

All individuals with COVID 19 were admitted to Millennium COVID-19 Care Center (MCCC) from August 1- October 30/2021.

4.4. Study Populations

All selected COVID 19 patients who were admitted and on follow-up at Millennium COVID-19 Care Center (MCCC) from August 1- October 30/2021 that fulfill the inclusion criteria.

4.5. Eligibility Criteria

4.5.1. Inclusion criteria

All adult (age > 18 years old) patients who were diagnosed with COVID-19 and had a complete blood count on the first day of admission were included in the study.

4.5.2. Exclusion Criteria

- Patients with an incomplete chart like, missed clinical and hematology data, and those without clinical outcomes data
- Patients who were referred from other centers after several days of hospitalization without attached admission complete blood count (CBC) in the first center and
- Those with hematological disorders like leukemia and lymphoma were excluded from the study.

4.6. Sample Size Determination

The sample size was determined by using single population proportion formula, with the assumption of incidence of severe COVID 19 is one in a five (20%) individuals with an underlying health condition from the study done on global, regional, and national estimates of the population (47). A 5% marginal error level of significance, 95 % CI with a total number of populations is 1092 in 3 month period (August, September and October) 2021 calendar year.

Hence, the sample size of the study was calculated as:

$$n = \frac{z^2 \times p(1 - p)}{d^2}$$

Where: n = desired sample size

α = the level of significance which can be obtained at 1- confidence level

P = best estimate of a population proportion

w = maximum acceptable difference

$Z_{\alpha/2}$ = the value under the standard normal table for the given value of confidence level So,

$$n = \frac{1.96^2 \times 0.20 \times 0.80}{d^2} = \frac{1.96^2 \times 0.2 \times 0.8}{0.05^2} = \frac{0.6144}{0.0025} = 246$$

But since the population is less than 10,000 finite populations, the correction formula was used to estimate the final sample size of the study.

$$\text{Therefore, } n_f = \frac{n}{1 + n/N} = \frac{246}{1.027} = 239.6$$

Where: n_f = final sample size

n = total sample size of the above formula and

N = total population

So, n_f = 240

For the second objective

By considering the case fatality rate of COVID 19 in Africa during the third wave period was 5.72% (48). A confidence level of 95% with 5% marginal error, the optimum sample size calculated with the above formula as follows;

$$n = \frac{1.96^2 \times 0.057 \times 0.94}{0.05^2} = 88$$

Based on the above two sample size calculation, 240 is the largest sample size, therefore, the final sample size of this study will be 240.

4.7. Sampling Procedure

According to Millennium COVID 19 center (MCC), the 3-month total number of COVID 19 was 1092. To select the first participant simple random sampling technique (lottery method) was used, and then every 4.5th interval was followed until the pre-determined sample sizes (240) were obtained. The sampling fraction (k^{th}) was stated as $k = (1092/240) = 4.5 \approx 4$, therefore every 4th patient was chosen as a study unit.

4.8. Study Variables

4.8.1. Dependent Variable

- In-hospital death (presence or absence of death)
- Sever form of COVID 19

4.8.2. Independent Variable

- Socio-demographic variables; Age, Sex, Residency place
- Characteristics on admission; Clinical symptoms, Vital signs, Comorbidity (Hypertension, Diabetes, Chronic kidney disease, Chronic Obstructive Pulmonary Disease, Human Immunodeficiency Virus)
- Laboratory findings;
 - WBC count with differential count, Hemoglobin, Platelet count, Neutrophil to Lymphocyte ratio
 - CXR, chest CT
 - BUN, Serum creatinine, ESR, AST, ALT, ALP
- Intervention; oxygen therapy, non-invasive ventilation, invasive ventilation, Renal replacement therapy, Vasopressors, Antibiotics, Steroid use, anticoagulation

4.9. Operational definition

COVID-19 severity score: was determined based on the WHO classification as follows (12).

Mild Disease: Characterized by fever, malaise, cough, upper respiratory symptoms, and/or less common features of COVID-19 (headache, loss of taste or smell, etc.)

Moderate Disease: Patients with lower respiratory symptom/s. They may have infiltrates on the chest X-ray. These patients can maintain oxygenation on room air.

Severe Disease: These patients have developed complications. The following features can define severe illness.

- Hypoxia: $SPO_2 < 93\%$ on atmospheric air or $PaO_2:FiO_2 < 300\text{mmHg}$
- Tachypnea: in respiratory distress or $RR > 30$ breaths/minutes
- Chest x-ray: more than 50% involvement seen on chest imaging

Critical COVID 19; Defined by the criteria for acute respiratory distress syndrome (ARDS), sepsis, septic shock, or other conditions that would normally require the provision of life-sustaining therapies such as mechanical ventilation (invasive or non-invasive) or vasopressor therapy.

4.10. Data Collection Procedures and tools

The patient medical records were reviewed by trained medical residents and general practitioners from Millennium COVID-19 Care Center (MCCC) using a standardized and pretested questionnaire.

Patient data was included demographics, medical history, exposure history, comorbidities, symptoms, laboratory findings, and treatment (oxygen, antibiotic, corticosteroid therapies, and respiratory support).

4.11. Data Management

A well-trained data collector and supervisor were carefully collected based on the inclusion criteria by an appropriately designed data collection instrument. The collected data were reviewed and checked for completeness on daily basis.

4.12. Data Analysis Procedure

The collected data were re-checked for completeness before the analysis process begins. The checked data was entered to SPSS version 26. Baseline characteristics were presented as numbers and percentages. Categorical variables were analyzed by cross tabulation and χ^2 test.

To assess the relationship between the NLR and in-hospital mortality and severity, the ROC curve was used and the report area under the ROC curve (AUC) was determined, then we regrouped the patients based on the NLR cut-off value for each severity and mortality. The odds ratio was calculated by comparing the occurrence of death or severity of disease on two different groups of

independent variables. In addition, to adjust potential confounding with variables, bivariate and multivariate logistic regression model was used.

Univariate analysis was done to screen out independent variables at a 25% level of significance, then incorporated into the analysis of multivariable binary logistic regression. For multivariable logistic regression, a 95% confidence interval for an adjusted odds ratio (AOR) was calculated and variables with a p-value ≤ 0.05 were considered as statistically associated with disease outcome and severity.

4.13. Ethical Consideration

Ethical clearance was obtained from the Institutional Review Board (IRB) of St. Paul Hospital Millennium Medical College. The officials at a different level in the study area were communicated through letters from St. Paul Hospital Millennium Medical College, Department of Internal Medicine. All data collected for this study were stored and confidentiality was maintained.

4.14. Dissemination of the result

After completion of the study, the result will be submitted to St. Paul Hospital Millennium Medical College, Department of Internal Medicine. Hard copies will be disseminated to Millennium COVID 19 care center, Ethiopian ministry of health, Ethiopian medical association, and Ethiopian internal medicine association. Also, manuscripts will be submitted for publication in peer-reviewed scientific journals and results will be presented in scientific conferences for better communication of the results.

5. Results

5.1. Socio-Demographic, Comorbidity and Presenting symptoms

A total of 240 COVID-19 patients with complete medical records were included in the current study. Among the 240 patients, 138(57.5%) of the study subjects were males. The majority (86.7%) of the participants were from Addis Ababa and the rest 13.3% were from different regions of Ethiopia; Oromia, Southern nation nationality of peoples and Amhara region.

According to the chief complaints of 240 patients, the most common symptoms at presentation were cough 142 (59.2%) and shortness of breath 63(26.3%). Less common symptoms were fever 14(5.8%) and fatigability, anosmia, loss of appetite, joint and muscle pain were identified in 21(8.8%) of cases. (Table 1)

Out of 240 patients with baseline comorbidity information, 145(60.4%) had a preexisting one or more co-morbid illnesses. The most prevalent comorbidities were hypertension 95(39.6%) and diabetes mellitus 83(34.6%), followed by Cardiac disease of 21(8.8%), COPD/Asthma 17(7.1%), CKD 14(5.8%), HIV/AIDS 8(4.2%) and Other comorbidities that include; chronic liver disease, dyslipidemia, polycythemia, hyperthyroidism, Parkinson's and alcoholic liver disease were account 12(5.4%). (Figure 2)

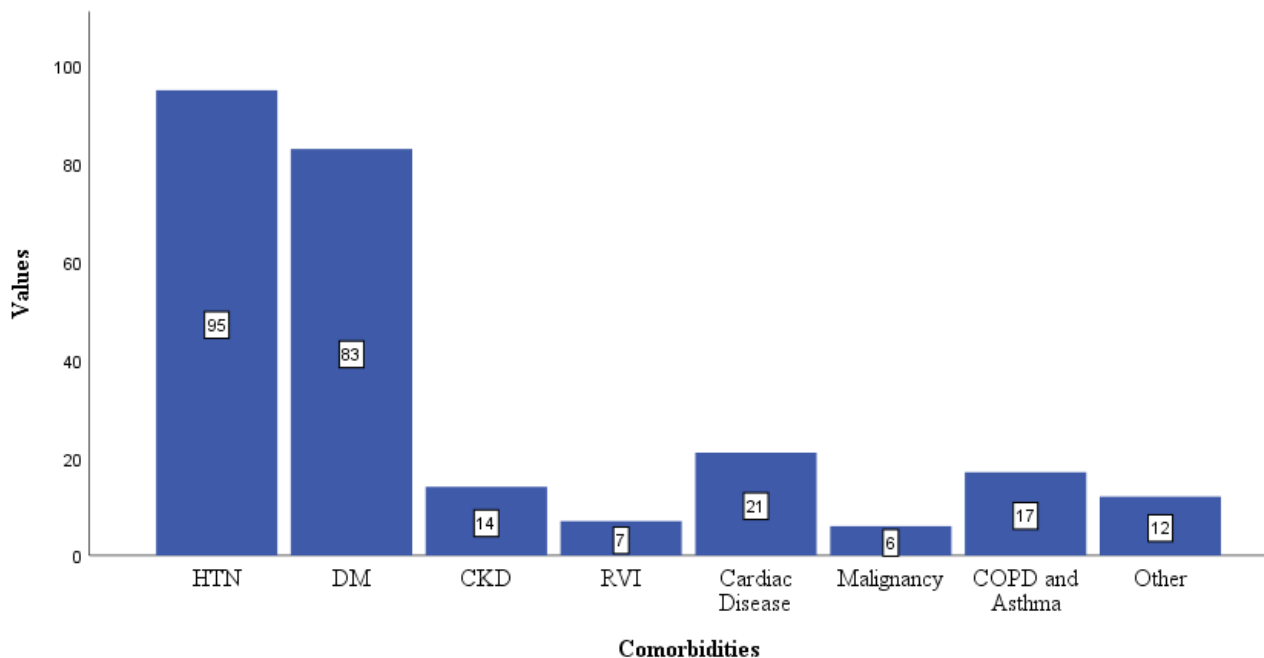


Figure 2: Pre-existing comorbidities among COVID 19 patients admitted at MCCC, Addis Ababa, Ethiopia, 2021

Table 1: Socio-demographic, co-morbid illness and presenting chief compliant among COVID-19 patients admitted at MCCC, Addis Ababa, Ethiopia, 2021

Variables		Frequency (%)
Age Group	<30	27(11.3)
	30-49	63(26.3)
	50-69	93(38.8)
	>70	57(23.8)
Gender	Male	138(57.5)
	Female	102(42.5)
Chief compliant	Cough	142(59.2)
	Shortness of breath	63(26.3)
	Fever	14(5.8)
	Fatigability, loss of appetite, loss of test and smell	16(6.7)
	Other (Headache, Arthralgia and myalgia)	6(2.5)
Comorbidity	NO	95(39.6)
	YES	145(60.4)
HTN	NO	145(60.4)
	YES	95(39.6)
DM	NO	157(65.4)
	YES	83(34.6)
CKD	NO	226(94.2)
	YES	14(5.8)
HIV/AIDS	NO	233(97.0)
	YES	7(2.9)
Cardiac disease	NO	219(91.3)
	YES	21(8.8)
Malignancy	NO	234(97.5)
	YES	6(2.5)
COPD and Asthma	NO	223(92.9)
	Yes	17(7.1)
Other	NO	228(95)
	YES	12(5)

Data are expressed as n (%).

Regarding the admission vital signs, patients had a mean heart rate of 94 ± 14 beats per minute and a mean respiratory rate of 25 ± 6 breath per minute. A quarter of patients were within the hypertensive systolic range and 20(8.3%) were in the hypertensive diastolic range. Body temperature of $>37.5^{\circ}\text{C}$ were 14(5.8%) and 116(48.3%) patients had an oxygen saturation of $\leq 93\%$ at the time of arrival to the treatment center. (Table 2)

Table 2: Vital sign at the presentation of COVID 19 patients admitted at MCCC, Addis Ababa, Ethiopia, 2021

Variables		Frequency (%)
SBP	<140mmHg	180(75)
	≥140 mmHg	60(25)
DBP	<90 mmHg	220(91.7)
	≥90 mmHg	20(8.3)
RR	<30breath/min	195(81.3)
	≥30breah/min	45(18.8)
PR	<100beat/min	155(64.6)
	≥100beat/min	85(35.4)
Temperature	≤37.5°C	226(94.2)
	>37.5 °C	14(5.8)
Saturation	>93%	124(51.7)
	≤93%	116(48.3)

Data are expressed as n (%).

5.2. Baseline laboratory finding

Regarding the laboratory tests, most 136(56.7%) of patients had normal white blood cell count with 40(16.7%) patients had leukopenia and 64(26.7%) had leukocytosis. The mean of absolute neutrophil and lymphocyte count was 6.0 ± 4.7 and 1.2 ± 0.6 , respectively and 129 (53.7%) of patients had lymphopenia. The majority 198(82.5%) of patients had normal platelet count with thrombocytopenia and thrombocytosis identified in 33(13.8%) and 9(3.8%) of cases, respectively.

More than two-thirds 187(78.2%) of patients had raised urea and 24(11.9%) had raised creatinine. Concerning liver enzymes, deranged levels of Serum Glutamic Oxaloacetic Transaminase (SGOT), Glutamic Pyruvic Transaminase (SGPT), and Alkaline Phosphatase (ALP) were identified on 95(40.6%), 66(28.4%), and 37(15.9%) of the patients, respectively. Among 83 diabetics' patients, we found 34 patients had documented HA₁C, of which 25(73.5%) patients had an HA₁C level of more than 6.5%. (Table 3)

Table 3: Baseline laboratory findings on the admission of COVID 19 patients admitted at MCCC, Addis Ababa, Ethiopia, 2021

Variables		Frequency (%)
White blood cell	<4.5 x10 ³ /μ l	40(16.7)
	4.5-11 x10 ³ /μ l	136(56.7)
	>11 x10 ³ /μ l	64(26.7)
Platelet	<150 x10 ³ /μ l	33(13.8)
	150-450 x10 ³ /μ l	198(82.5)
	>450 x10 ³ /μ l	9(3.8)
Hemoglobin	<12 mg/dl	37(15.4)
	12-15 mg/dl	119(49.6)
	>15 mg/dl	84(35.0)
Hematocrit	<36%	44(18.3)
	36-45%	128(53.3)
	>45%	68(28.3)
BUN	<20mg/dl	52(21.8)
	>20mg/dl	187(78.2)
Creatinine	<0.6mg/dl	62(30.8)
	0.6-1.1mg/dl	115(57.2)
	>1.2mg/dl	24(11.9)
ALT/GPT	<40 IU/l	166(71.6)
	>40 IU/l	66(28.4)
AST/GOT	<40 IU/l	139(59.4)
	>40 IU/l	95(40.6)
ALP	<130 IU/l	196(84.1)
	>130 IU/l	37(15.9)
Total Bilirubin	<0.2 mg/dl	19(15.3)
	0.2-1.2 mg/dl	95(76.6)
	>1.2 mg/dl	10(8.1)
HA1C	≥6.5%	25(73.5)
	<6.5%	9(26.5)

Data are expressed as n (%).

5.3. Imaging finding of COVID 19 patients

Among 107 patients who underwent baseline CXRs upon presentation and throughout their hospital stay, Eighty-six of 107 patients demonstrated abnormalities on CXR, nearly half of patients 53(49.5%) had ground-glass opacification (GGO) and the remaining 43(40.2%) had consolidation. With regards to the overall CXR distribution of abnormal findings, peripheral shadowing was present in 88(91.7%) followed by diffuse distribution pattern 7(7.3%). Bilateral involvement of lung

field was a common finding that accounts in 86(89.6%) of cases. Similarly, out of 18 patients identified with chest CT, Eleven (61.1%) patients had ground-glass opacification (GGO). The major 14(78.4%) pattern of distribution was peripheral and diffuse (central and peripheral) lung changes were noted in 7 patients (7.3%). Bilateral middle and lower lung zone involvements were noted in 12 patients (66.7%). (Table 4)

Table 4: Imaging finding of COVID 19 patients admitted at MCCC, Addis Ababa, Ethiopia, 2021

Variables		Frequency (%)
CXR opacity	GGO	53(49.5)
	Consolidation	43(40.2)
	Unremarkable	11(10.3)
CXR Distribution	Peripheral predominant	88(91.7)
	Perihillar predominant	1(1.0)
	Diffuse	7(7.3)
CXR Location	Bilateral	86(89.6)
	Right lung	6(6.3)
	Left Lung	4(4.2)
Chest CT Density	GGO	11(61.1)
	Consolidation	7(38.9)
Chest CT Distribution	Peripheral	14(77.8)
	Diffuse	4(22.2)
Chest CT Location	Bilateral middle and lower lobe	14(77.8)
	Bilateral and diffuse	4(22.2)

Data are expressed as n (%).

5.4. Severity and disease outcome of COVID-19

A total of 240 patients with COVID-19 were studied, Almost half the patients 116(48.3%) had severe disease (38.8% severe and 9.6% critical) and the rest 124(51.6%) had non-severe diseases (27.9% mild and 23.8% moderate) at the time of admission. Regarding disease outcome, a quarter (25.85%) of patients died. (Figure 3)

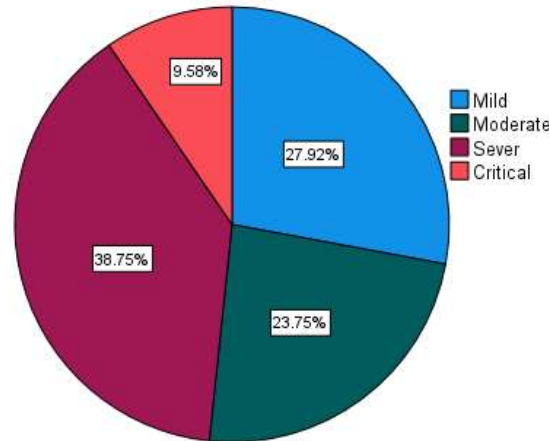


Figure 3: Severity of COVID 19 patients admitted at MCCC, Addis Ababa, Ethiopia, 2021

5.5. In-hospital COVID 19 related complication, treatment option and intervention

Although three-quarters 169(71.2%) of patients did not develop any complications, 71(29.5%) of patients had a complication with the most commonly mentioned complication were concomitant occurrence of infectious and coagulopathy related complications in 33(13.8%) of cases, whereas the remaining 25(10.4%) had isolated infectious related (ARDS, AKI, HAI, Sepsis), 9(3.8%) isolated coagulopathy complication (PE, PTE, DVT) and Four patients (1.7%) cases had an acute cardiac injury.

Concerning the management of COVID 19, most 203(84.6%) of the patients received one or more antibiotics before admission to the center and throughout their stay in the hospital, regarding the choice of antibiotics ceftriaxone 144(70.9%) and azithromycin 152(74.8%) were the most commonly used drugs in the treatment center followed by vancomycin, ceftazidime, cefepime, and meropenem were used in 57(28%), 49(24.1%), 10(4.9%) and 5(2.4%) of cases, respectively. (Figure 3)

Among the participants, Three-quarters 117(73.8%) of patients received steroids, nearly one-fourth 55(22.9%) of the patient received vasopressor therapy, sixthly-six (27.5%) patients also took anticoagulant therapy for coagulopathy related complication and presumed pulmonary thromboembolism (PTE). Concerning the need for intervention, one-fourth 60(25%) of the patients needed respiratory support, three patients (1.3%) underwent one or more hemodialysis sessions and Four patients (1.6%) had received a blood transfusion for their underlying illness. (Table 5)

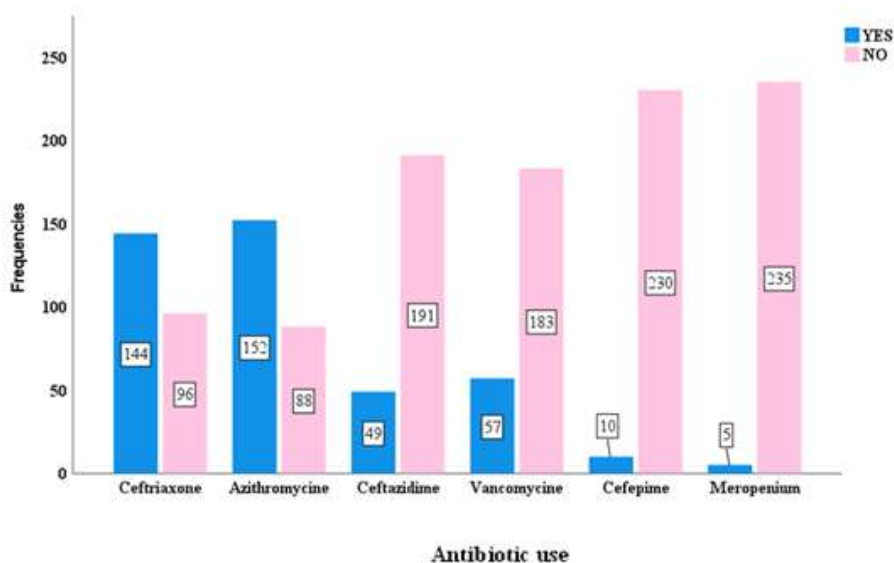


Figure 4: Distribution of Antibiotics use in COVID 19 patients admitted at MCCC, Addis Ababa, Ethiopia, 2021

Table 5: In-hospital COVID 19 related complication, treatment option and intervention among patients admitted at MCCC, Addis Ababa, Ethiopia, 2021

Variables		Frequency (%)
Antibiotics use	NO	37(15.4)
	YES	203(84.6)
Steroid	NO	63(26.3)
	YES	177(73.8)
Vasopressor	NO	185(77.1)
	YES	55(22.9)
Anticoagulant	NO	174(72.5)
	YES	66(27.5)
RRT	NO	237(98.8)
	YES	3(1.3)
MV	NO	180(75)
	YES	60(25)
Complication	NO complication	169(70.4)
	Infectious and Coagulopathy related	33(13.8)
	AKI, ARDS, HAI, Sepsis	25(10.4)
	Coagulopathy: PE,PTE,DVT	9(3.8)
	Acute Cardiac Injury	4(1.7)

Data are expressed as n (%).

5.6. ROC curve Analysis

The ROC curves were analyzed with the SPSS software version 26 and were plotted for NLR for mortality and severity of COVID 19. The analysis of the ROC curve illustrated a 0.95 area under the curve (AUC) for NLR levels as a predictor of in-hospital mortality (95% CI: 0.92-0.98, $P < 0.0001$). The AUC of this biomarker indicated a high predictive value for the outcome, with the optimal threshold value being 9.47 with a sensitivity of 88.7% and a specificity of 95.40%, the corresponding positive and negative predictive values were 62.5% and 95.4% respectively. (Figure 5a)

Regarding the ROC curve for the severity of COVID 19, NLR of 5.86 was demonstrated as the optimal cut-off value of predictor of severe disease with the sensitivity of 92.2% and a specificity of 75% (area under the curve (AUC): 0.858, 95%CI 0.80-0.90; $P < 0.0001$), in the same fashion the corresponding positive and negative predictive values were 77.5% and 91% respectively. (Figure 5b)

Table 6: ROC value for inflammatory markers for predicting COVID 19 mortality and severity

Variables	AUC	Cutoff	Sensitivity	Specificity	PPV	NPV
NLR for severity	0.85	5.86	92.20%	75%	77.50%	91%
NLR for mortality	0.95	9.47	88.70%	95.40%	62.50%	95.40%

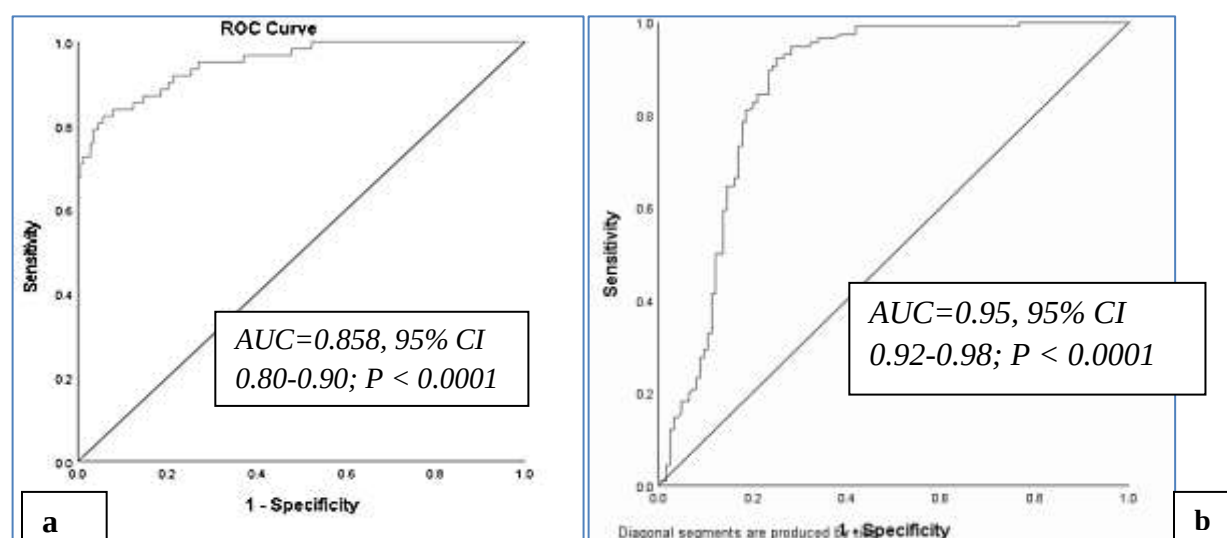


Figure 5; ROC curves of neutrophil to lymphocyte ratios in predicting mortality (a) and severity (b) of patients with COVID-19.

5.7. Comparison of Socio demography, Comorbid illness, vital-signs and laboratory variables between Non-severe with Severe and survivor with non-survivor groups in COVID-19 patients.

A higher proportion of COVID-19 disease severity was observed among the groups classified by Age 50-69, age >70, presence of shortness of breath, presence of comorbidity, and presence of HTN, DM and cardiac disease. Among the vital sign and laboratory markers; high respiratory rate, low oxygen saturation, leucocytosis, thrombocytosis, raised ANC, lymphopenia, elevated BUN, and deranged both GOT and GPT were observed in large proportion severe group. (Tables 7 and 8)

Table 7: Comparison of Socio demography and Comorbid illness variables between Non-severe with Severe and survivor with non-survivor groups in COVID-19 patients at MCCC, Addis Ababa, Ethiopia, 2021.

Variables		Outcome		Severity	
		Survivor n (%)	Death n (%)	Non-Sever n (%)	Sever n (%)
Age group	<30	25(92.6)	2(7.4)	22(81.5)	5(18.5)
	30-49	52(82.5)	11(17.5)	38(60.3)	25(39.7)
	50-69	65(69.9)	28(30.1)	42(45.2)	51(54.8)
	>70	36(63.2)	21(36.8)	22(38.6)	35(61.4)
Gender	Male	107(77.5)	31(22.5)	70(50.7)	68(49.3)
	Female	71(69.6)	31(30.4)	54(52.9)	48(47.1)
Chief Complaint	Cough	110(77.5)	32(22.5)	75(52.8)	67(47.2)
	Shortness of breath	41(65.1)	22(34.9)	19(30.2)	44(69.8)
	Fever	12(85.7)	2(14.3)	13(92.9)	1(7.1)
	Fatigability, loss of test and smell	12(75)	4(25)	12(75)	4(25)
	Headache, arthralgia and myalgia	3(60%)	2(40%)	5(100%)	0(0%)
Comorbidity	NO	84(88.4)	11(11.6)	55(57.9)	40(52.1)
	YES	94(64.8)	51(35.2)	69(47.6)	76(52.4)
HTN	NO	122(84.1)	23(15.9)	86(59.3)	59(40.7)
	YES	56(58.9)	39(41.1)	38(40)	57(60)
DM	NO	127(80.9)	30(19.1)	89(56.7)	68(43.3)
	YES	51(61.4)	32(38.6)	35(42.2)	48(57.8)
CKD	NO	170(75.2)	56(24.8)	113(50)	113(50)
	YES	8(57.1)	6(42.9)	11(78.6)	3(21.4)
HIV/AIDS	NO	174(75.7)	56(25.3)	120(51.5)	113(48.5)
	YES	4(40.0)	3(42.9)	4(57.4)	3(42.9)
Cardiac Disease	NO	163(74.4)	56(25.6)	114(52.1)	105(47.9)
	YES	15(71.4)	6(28.6)	10(47.6)	11(52.4)
Malignancy	NO	174(74.4)	60(25.6)	119(50.9)	115(49.1)
	YES	4(66.7)	2(33.3)	5(83.3)	1(16.7)
COPD and Asthma	NO	166(74.4)	57(25.6)	115(51.6)	108(48.4)
	Yes	12(70.6)	5(29.4)	9(52.9)	8(47.1)

Data are expressed as n (%).

Table 8: Laboratory biomarker, vital sign related variables and comparison between Non-severe with severe and survivor with non Survivors of COVID-19 patients among patients admitted at MCCC, Addis Ababa, Ethiopia, 2021

Variables		Outcome		Severity	
		Survivor n (%)	Death n (%)	Non-Sever n(%)	Sever n (%)
SBP	<140mmHg	137(76.1)	43(23.9)	100(55.6)	80(44.4)
	≥140mmHg	41(68.3)	19(31.7)	24(40)	36(60)
DBP	<90mmHg	168(76.4)	52(23.6)	118(53.6)	102(46.4)
	≥90mmHg	10(50)	10(50)	6(30)	14(70)
Temperature	≤37.5°C	167(73.9)	59(26.1)	113(50)	113(50)
	>37.5°C	11(78.6)	3(21.4)	11(78.6)	3(21.4)
RR	<30breath/min	152(77.9)	43(22.1)	117(60)	78(40)
	≥30breath/min	26(57.8)	19(42.2)	7(15.6)	38(84.4)
PR	≥100beat/min	116(74.8)	39(25.2)	84(54.2)	71(45.8)
	>100breath/min	62(72.9)	23(27.1)	40(47.1)	45(52.9)
SO ₂	>93%	81(65.3)	43(34.7)	9(7.3)	115(92.7)
	≤93%	97(83.6)	19(16.4)	0(0%)	116(99.1)
White blood cell	<4.5 x10 ³ /μl	36(90)	4(10)	33(82.5)	7(17.5)
	4.5-11x10 ³ /μl	114(83.8)	22(16.2)	77(56.6)	59(43.4)
	>11 x10 ³ /μl	28(43.8)	36(56.3)	14(21.9)	50(78.1)
Platelet	<150 x10 ³ /μl	22(66.7)	11(33.3)	20(60.6)	13(39.4)
	150-450 x10 ³ /μl	150(75.8)	48(24.2)	101(51.0)	97(49.0)
	>450 x10 ³ /μl	6(66.7)	3(33.3)	3(33.3)	6(66.7)
Hemoglobin	<12 mg/dl	18(48.6)	19(51.4)	25(67.6)	12(32.4)
	12-15 mg/dl	87(73.1)	32(26.9)	56(47.1)	63(52.9)
	>15 mg/dl	73(86.9)	11(13.1)	43(51.2)	41(48.8)
Hematocrit	<36%	23(52.3)	21(47.7)	28(63.6)	16(36.4)
	36-45%	102(79.7)	26(20.3)	64(50)	64(50)
	>45%	53(77.9)	15(22.1)	32(47.1)	36(52.9)
ANC	<6.5 x10 ³ /μl	115(93.5)	8(6.5)	91(74)	32(26)
	>6.5 x10 ³ /μl	63(53.8)	54(46.2)	33(28.2)	84(71.8)
ALC	>1.1 x10 ³ /μl	107(96.4)	4(3.6)	87(78.4)	24(21.6)
	<1.1 x10 ³ /μl	71(55)	58(45)	37(28.7)	92(71.3)
BUN	<20 mg/dl	45(86.5)	7(13.5)	37(71.2)	15(28.8)
	>20mg/dl	133(71.1)	54(28.9)	87(46.5)	100(53.5)
Creatinine	<0.6 mg/dl	47(75.8)	15(24.2)	36(58.1)	26(41.9)
	0.6-1.1mg/dl	91(79.1)	24(20.9)	57(49.6)	58(50.4)
	>1.2mg/dl	12(50)	12(50)	13(54.2)	11(45.8)
ALT/GPT	<40 IU/l	125(75.3)	41(24.7)	94(56.6)	72(43.4)
	>40 IU/l	48(72.7)	18(27.3)	26(39.4)	40(60.6)
AST/GOT	<40 IU/l	108(77.7)	31(22.3)	86(61.9)	53(38.1)
	>40 IU/l	67(70.5)	28(29.5)	36(37.9)	59(62.1)
ALP	<130 IU/l	152(77.6)	44(22.4)	104(53.1)	92(46.9)
	>130 IU/l	22(59.5)	15(40.5)	18(48.6)	19(51.4)
Total Bilirubin	<0.2mg/dl	18(94.7)	1(5.3)	10(52.6)	9(47.4)
	0.2-1.2mg/dl	68(71.6)	27(28.4)	47(49.5)	48(50.5)
	>1.2mg/dl	9(90)	1(10)	5(50)	5(5)
Direct Bilirubin	<0.3mg/dl	87(72.5)	33(27.5)	64(53.3)	56(46.7)
	>0.3mg/dl	10(66.7)	5(33.3)	7(46.7)	8(53.3)

5.8. Factors that predict the mortality of patients with COVID 19

In univariate logistic regression, 25% level of significance ($P < 0.25$) was conducted some factors were found significantly associated and predict the outcome (in-hospital death). These factors were: Age greater than 65, sex, presence of comorbidity, presence of Hypertension, presence of diabetes, presence of CKD, and presence of HIV/AIDS.

Among vital signs and laboratory markers that affect in-hospital death, the following factors were; respiratory rate above 20, systolic and diastolic hypertension, low hemoglobin concentration, high WBC count, high absolute neutrophil count, low absolute lymphocyte count, raised creatinine, high aspartate aminotransferase, and high NLR had significant association on crude analysis at 25% of significance.

However, based on the multivariable binary logistic regression model at a 5% level of significance, leukocytosis, lymphopenia, anemia, raised ANC and NLR of > 9.47 were found significant association with the disease outcome. (Table 9)

Table 9: Univariate and multivariate analysis for risk factors associated with mortality patients with COVID-19 (n=240)

Variables		Outcome		COR(95% CI)	AOR(95%CI)	P-Value
		Survivor n (%)	Death n (%)			
Age	<65	138(78.9)	37(21.1)	1	1	
	≥65	40(61.5)	25(38.5)	2.331(1.257-4.323)	1.279(0.414-3.952)	0.668
Gender	Male	107(77.5)	31(22.5)	1	1	
	Female	71(69.6)	31(30.4)	1.507(0.843-2.695)	1.678(0.603-4.672)	0.322
Comorbidity	NO	84(88.4)	11(11.6)	1	1	
	YES	94(64.8)	51(35.2)	4.143(2.027-8.469)	0.713(0.112-4.534)	0.721
HTN	NO	122(84.1)	23(15.9)	1	1	
	YES	56(58.9)	39(41.1)	3.694(2.018-6.762)	3.266(0.840-12.702)	0.088
DM	NO	127(80.9)	30(19.1)	1	1	
	YES	51(61.4)	32(38.6)	2.656(1.466-4.814)	1.855(0.487-7.073)	0.365
CKD	NO	170(75.2)	56(24.8)	1	1	
	YES	8(57.1)	6(42.9)	2.276(0.757-6.845)	0.236(0.013-4.238)	0.328
HIV/AIDS	NO	174(75.7)	56(25.3)	1	1	
	YES	4(40.0)	3(42.9)	4.660(1.270-17.110)	17.582(0.811-381.19)	0.068
SBP	<140mmHg	137(76.1)	43(23.9)	1	1	
	>140mmHg	41(68.3)	19(31.7)	1.476(0.776-2.808)	1.548(0.487-4.920)	0.459
DBP	<90mmHg	168(76.4)	52(23.6)	1	1	
	>90mmHg	10(50)	10(50)	3.230(1.275-8.189)	1.430(0.275-7.443)	0.671
RR	<20breath/min	49(80.3)	12(19.7)	1	1	
	>20breath/min	129(72.1)	50(27.9)	1.582(0.778-3.221)	0.611(0.168-2.228)	0.456
WBC	<4.5, 4.5-11	150(85.2)	26(14.8)	1		
	>11 x10 ³ /μl	28(43.8)	36(56.3)	7.417(3.888-14.151)	3.607(1.017-12.798)	0.047*
ANC	<6.5 x10 ³ /μl	115(93.5)	8(6.5)	1	1	
	≥6.5 x10 ³ /μl	63(53.8)	54(46.2)	12.321(5.517-27519)	3.840(0.841-17.547)	0.083
ALC	>1.1 x10 ³ /μl	107(96.4)	4(3.6)	1	1	
	≤1.1 x10 ³ /μl	71(55)	58(45)	21.852(7.596-62.865)	12.939(3.392-49.352)	0.0001*
NLR	<9.47	145(95.4)	7(4.6)	1	1	
	>9.47	33(37.5)	55(62.5)	34.523(14.425-82.62)	4.735(1.194-18.778)	0.027*
HGB	>13mg/dl	102(76)	20(16.4)	1	1	
	<13mg/dl	76(64.4)	42(35.6)	2.818(1.532-5.186)	3.500(1.154-10.621)	0.027*
Creatinine	<1.1mg/dl	164(78.1)	46(21.9)	1	1	
	>1.2mg/dl	12(50)	12(50)	3.565(1.502-8.462)	3.487(0.615-19.761)	0.158
AST/GOT	<40 Iu/l	108(77.7)	31(22.3)	1	1	
	>40 Iu/l	67(70.5)	28(29.5)	1.455(0.803-2.640)	1.634(0.589-4.536)	0.346

Data are expressed as n (%). 1= reference

*p-value <0.05(on multivariate analysis)

5.9. Factors influencing the severity of patients with COVID 19

Several factors were found to be significantly associated with disease severity, in univariate binary logistic regression, at a 25% level of significance ($P < 0.25$), these factors include: Age greater than 65, presence of diabetes, hypertension, malignancy, a respiratory rate greater than 30, elevated blood pressure, increase in WBC, increase in NLR, increase absolute neutrophil count, low absolute lymphocyte count, raised urea and deranged liver enzymes (GOT and GPT).

However; only high NLR(>5.89), decrement in absolute lymphocyte count, and elevated level of GOT/AST were found to be significantly associated with disease severity in the multivariable binary logistic regression model at a 5% level of significance. (Table 10)

Table 10: Univariate and multivariate analysis for risk factors associated with severity of disease with COVID-19 (n=240)

Variables		Severity		COR(95%CI)	AOR(95%CI)	P-Value
		Non-Sever n (%)	Sever n(%)			
Age	<65	101(57.7)	74(42.3)	1	1	
	≥65	23(35.4)	42(64.6)	2.492(1.381-4.497)	1.345(0.544-3.326)	0.521
Comorbidity	NO	55(57.9)	40(52.1)	1	1	
	YES	69(47.6)	76(52.4)	1.514(0.889-2.552)	0.391(0.108-1.422)	0.154
HTN	NO	86(59.3)	59(40.7)	1	1	
	YES	38(40)	57(60)	2.186(1.290-3.705)	1.954(0.691-5.527)	0.206
DM	NO	89(56.7)	68(43.3)	1	1	
	YES	35(42.2)	48(57.8)	1.794(1.048-3.074)	1.054(0.381-2.920)	0.919
Malignancy	NO	119(50.9)	115(49.1)	1	1	
	YES	5(83.3)	1(16.7)	0.206(0.024-1.799)	0.356(0.017-7.377)	0.505
SBP	NO	100(55.6)	80(44.4)	1	1	
	YES	24(40)	36(60)	1.874(1.035-3.397)	2.367(0.849-6.599)	0.100
DBP	NO	118(53.6)	102(46.4)	1	1	
	YES	6(30)	14(70)	2.699(1.001-7.282)	0.854(0.208-3.514)	0.828
WBC	<4.5, 4.5-11	110(62.5)	66(37.5)	1	1	
	>11 x10 ³ /μl	14(21.9)	50(78.)	16.836(6.143-46.14)	1.320(0.453-3.853)	0.611
HGB	>13mg/dl	61(50)	61(50)	1	1	
	<13mg/dl	63(53.4)	55(46.6)	1.087(0.526-1.45)	0.556(0.242-1.282)	0.169
ANC	<6.5 x10 ³ /μl	91(74)	32(26)	1	1	
	>6.5 x10 ³ /μl	33(28.2)	84(71.8)	7.238(4.095-12.79)	2.692(0.948-7.645)	0.063
ALC	>1.1 x10 ³ /μl	87(78.4)	24(21.6)	1	1	
	<1.1 x10 ³ /μl	37(28.7)	92(71.3)	9.014(4.989-16.28)	3.030(1.076-8.53)	0.036*
NLR	<5.89	93(91.2)	9(8.8)	1	1	
	>5.89	31(22.5)	107(77.5)	35.67(16.148-78.7)	12.98(3.846-43.80)	0.0001*
AST/GOT	<40Iu/l	86(61.9)	53(38.1)	1	1	
	>40 Iu/l	36(37.9)	59(62.1)	2.659(1.554-4.55)	2.666(1.109-6.410)	0.028*
ALT/GPT	<40Iu/l	94(56.6)	72(43.4)	1	1	
	>40 Iu/l	26(39.4)	40(60.6)	2.009(1.123-3.59)	1.097(0.420-2.871)	0.849
BUN	<20mg/dl	37(71.2)	15(28.8)	1	1	
	>20mg/dl	87(46.5)	100(53.5)	2.835(1.458-5.51)	1.140(0.415-3.139)	0.798

Data are expressed as n (%). 1= reference

*p-value <0.05(on multivariate analysis)

6. Discussion

In the current study, we conducted a retrospective cross-sectional study to evaluate the predictive potential of NLR as a predictor of mortality and severity of disease among 240 COVID 19 patients who were admitted to Millennium COVID-19 Care Center in Ethiopia from August 1 to October 30, 2021. Out of 240 patients were studied, almost half of patients 116(48.3%) had severe disease and the rest 124(51.6%) had non-severe disease. Regarding the disease outcome, we found that a quarter 62(25.8%) of patients died and 178(74.2%) were discharged alive.

Based on the ROC curve analysis, The optimal cut-off level of NLR for mortality was 9.47 which is concordant with the retrospective observational study of Romania and Iran where the optimal cutoff value of 9.1 and 9.0 respectively, however, our result is slightly higher than 7.9 and lower than 11.75 of two studies done in China (27,29,49,50).

Regarding the severity of disease, we suggested NLR of 5.89 as an optimal cut-off value for severity, which is in line with one study done in China (51), and nearly close to two reports from Iran and China were cut off value of 6.5 and 4.5, respectively (29,52), but the value was higher than 3.3, 3.13 and 4.06 of three studies from China and (31,33,53), this difference might be attributed to the use of different dependent variables such as the likelihood of admission to ICU, developing shock and likelihood underwent intubation were used to define sever COVID-19 that might produce different cut off value among studies.

In addition, this variation might indicate that absolute NLR values measured in different populations are hardly comparable and optimal cut-off values may vary from one population to another, and lack of data on the reference range in a specific population may also be contributed to these differences. Therefore, determining the cut-off value is essential for NLR to be used in clinical settings of a specific locality.

The current study identified an NLR cutoff value of ≥ 9.47 for mortality with demonstrated sensitivity and specificity of 88.7% and 95.4%, respectively and the positive predictive value was 62.5% while the negative predictive value was 95.4%.which is in proximity to the pooled sensitivity (83%) and pooled specificity (83%) of a meta-analysis of ten studies involving 2967 patients with the cut-off value of 6.5 (54).

When we compared with the previous studies; the Romania study recommends 9.1 as a cut-off value for NLR with 70% sensitivity and 67% specificity for mortality (50). Two studies performed a ROC analysis to determine the optimal cutoff value of 11.75 with a sensitivity of 97.5% and a specificity of 78.1% (27) and another study provide a cut-off value of 7.945 with a sensitivity of 65.3%, and a specificity of 90.6% (49), this narrow range variability might be due to different optimal cut-off value used in different literature, different conditions of patients or different comorbidities among the included study subjects.

The present study also revealed that the NLR of ≥ 5.89 cut-off value for the severity of disease with sensitivity and specificity of NLR was 92.2% and 75%, respectively, and PPV of 75.5% and NPV of 91%. This is consistent with the pooled specificity (78%), but higher sensitivity value than pooled sensitivity (78%) of meta-analysis of thirteen studies involving 1579 patients at a cut-off value of 4.5 (54).

Similarly, retrospectively analyzed clinical data from China revealed that optimal threshold of 4.795 with a sensitivity of 83.9% and a specificity of 75% in line with our study with some narrow range difference in sensitivity (55), this gap might be related to the use of variable cut-off value and condition of the patient.

Based on the multivariable logistic regression analysis at a 5% level of significance, Our study supports that a high NLR at hospital admission was a strong independent factor for in-hospital mortality in patients with COVID-19. The risk of in-hospital death was 4.735 times higher in patients with NLRs ≥ 9.45 than those patients having NLR of < 9.45 with 95% CI, 1.19-18.78, $P=0.027$. This Odds ratio falls in the range between 1.8 to 44 of odds of mortality that reported in previous studies in respective of different cut-off values (26,27,32,56), in addition, it is nearly comparable with a report from a meta-analysis which revealed that odds of mortality increased by 2.74 fold in patients with elevated NLR than those with normal NLR (56).

Correspondingly, COVID-19 patients with an NLR of 5.89 on admission had significantly higher odds of severe disease as well as a strong prognostic factor for increased morbidity with an adjusted odds ratio (AOR) of 12.98, 95%CI, 3.85-43.80; $P<0.001$. This is in line with the diagnostic odds ratio (DOR) of 13 with a cut-off value of 4.5 and it is closely related DOR of 11.45 with a cut-off value of 3.63 of two meta-analyses that include 13 and 10 articles respectively (17,54).

This persistent association might be due to the pathogenesis of SARS CoV that result in extensive infiltration of neutrophils in the lung and increased neutrophils in the peripheral blood and with growing evidence of a decrease in lymphocyte number by SARS CoV virus due to increased pro-inflammatory mediators that bind to lymphocytic surface receptors and subsequently initiate lymphocytic apoptosis leading to lymphopenia that predisposes severe COVID-19 patients to cytokine storm, thus leading to more lymphocytic apoptosis and multi-organ failure (17,54,56,57).

Lymphopenia less than $1.1 \times 10^3/\mu\text{l}$ at admission was found to be an independent factor that significantly associated with increased odds of progression to severe disease (AOR=3.031, 95%CI, 1.08-8.53, $P=0.036$) and odds of in-hospital mortality (AOR= 12.94, 95% CI, 3.39-49.35, $P \leq 0.001$). This is in agreement with meta-analysis that showed an increased odds of severity by 3.7fold (58), and the odds of mortality also incongruent with a subgroup analysis of systematic review in which the odds of mortality increased by 12 fold, even though the cut-off value was 500 cell/L (severe lymphopenia) (59). Likewise, our finding is not far from a report of another meta-analysis that increased the odds of mortality by 9 fold (60). This acceptable variability might be the use of different reference values among the studies and the type of methodology used, along with a common denominator of speculated potential mechanisms that lead to lymphocyte deficiency which are; death of infected lymphocytes, lymphocytic dysfunction, inflammatory cytokines leading to lymphocyte apoptosis and inhibition of lymphocytes by metabolic molecules produced by metabolic disorders, such as hyperlactic acidemia (17,27,56,57).

Other laboratory markers that are found to be significant determinants of disease outcome were raised white blood cell count and low hemoglobin concentration at admission. After being adjusted for other factors, having Leukocytosis above $11 \times 10^3/\mu\text{l}$ at admission was associated with 3.607 times higher odds of dying compared to those with normal values and leukopenia. This is consistent with a meta-analysis of 34 studies and other previous studies done in Ethiopia and China (61–63). A raised white cell count is an indication of hyperinflammation and a strong immune system responding to a pathogenic organism.

Similarly, having a low hemoglobin concentration was associated with 3.5 times higher odds of dying compared to those with hemoglobin values of 13 and above, which is in line with a study done in Austria that increased mortality by 3.7 fold (64) and is nearly comparable with a meta-analysis of 5 studies and other retrospective studies done in the USA and a prospective study done

in Iran (65–67). Indeed, anemia can further reduce oxygen delivery to peripheral tissue in COVID-19 patients who have an increased oxygen demand due to interstitial pneumonia.

The risk of developing a severe disease among patients with deranged values of serum GOT/AST was 2.666 times that of patients with a value of the normal range, which is coherent with a recent systemic review and cross-sectional study (68,69). A potential cause of the hepatic injury may be multifactorial which is related to the direct effect of the virus and systemic effects of COVID-19 sepsis leads to hypoxic damage and liver ischemia.

7. Strengths and Limitations

The results of this study have several clinical implications and strengths. Our data confirms and expands previous results in COVID-19 patients and emphasizes the relevance of routine hematologic tests and provided a simple way to determine the poor prognosis of patients with COVID-19, which is conducive to the allocation of medical resources in our limited resource setup.

There are several limitations to the study;

1. Due to the single-center, retrospective nature of the study design and limited sample size, the real value of the NLR might be underestimated or overestimated in predicting poor prognosis, so the conclusions may not be generalizable to other hospitals. Further prospective multicenter studies that involve different regions are needed to ascertain the real value of the NLR and also needed to validate prospectively in the same center.
2. Since all subjects in our study were hospitalized patients diagnosed with COVID-19, the results of this study might not directly be applied to other ambulatory patients.
3. Although we have adjusted for multiple potential confounders, residual and unmeasured confounding such as smoking history, BMI, coagulation predictors, and prior exposure of patients to different medications like steroids which are missed from patient records due to the retrospective nature of the study.
4. We only analyzed NLR at admission; however, there may be a need to be serial monitoring of NLR throughout the disease course and its response to different treatment strategies, which could have more specificity in predicting mortality and severity of the disease.
5. Our study lacked a comparison of NLR with other well-known inflammatory markers like CRP and D-dimer.

8. Conclusions

In conclusion, NLR is a simple inflammatory biomarker that reflects the presence of systemic inflammation and is easily detected at admission, $\text{NLR} \geq 9.47$ could be utilized as a predicting indicator for the early recognition of COVID-19 mortality, and $\text{NLR} \geq 5.86$ was identified as predicting indicator for severe form COVID-19. Values above these thresholds were significantly associated with all-cause of COVID-19 related mortality and severity of the disease. It provides an objective input for early decision-making in the patient's management and allocation of limited resources like mechanical ventilation.

9. Recommendation

For Health professionals

- The clinician should consider baseline NLR as one of the inflammatory markers to predict the mortality and severity of COVID-19 and apply to on their patients for early identification and timely treatment modification to reduce in-hospital death.
- Since there is heterogeneity in cut-off value among studies, a clinician who works at MCCC can take our optimal cut-off value as a surrogate reference along with other clinical factors.

For Minister of health/Addis Ababa health bureau

- It's recommended to take this biomarker as input for allocation of limited resources like mechanical ventilation when large volume cases occur at the time.

For researchers

- Future studies may be needed to validate our result prospectively on the same center and multicenter study with a larger sample size by including additional confounding factors and dynamic monitoring of NLR in the course of the disease are recommended.
- Further studies are also required to assess whether NLR would result in a better predictive value compared with other expensive inflammatory biomarkers.

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11. Annex I

11.1 Data Collection Format

The Purpose of this study is to evaluate the predictive values of NLR on severity and mortality in patients with COVID-19 admitted in millennium COVID-19 treatment centers Addis Ababa, Ethiopia,

I kindly request your willingness to perform these reviews. The information gathered will be used confidentially.

Code	Question	Response	Remark
Part one: Socio-demography factors			
SD1	MRN	_____	
SD2	Age	_____	
SD3	Sex	Male	
		Female	
SD4	Residency	_____	
SD5	Marital status	Single	
		Married	
		Divorced	
		Separated	
		Widowed	
SD6	Occupation	_____	
Part two: Clinical characteristics			
CC1	Chief complaint	_____	
CC2	History of Smoking	Yes	
		NO	
CC3	Comorbidity	HTN	
		DM	
		COPD	
		CKD	
		HIV	
		OTHER_____	
CC4	Vital sign	BP_____	
		PR_____	
		RR_____	
		T ^o _____	
		So ₂ _____	
		BMI_____	

Part three: Laboratory finding and Imaging			
LF1	CBC at admission	WBC	
		Neutrophil No	
		Lymphocyte No	
		NLR	
		N%	
		L%	
		Hgb	
		PLT	
LF2	RFT	BUN	
	Creatinine		
LF3	LFT	ALT	
		AST	
		ALP	
		Total BIL	
		ID BIL	
		Albumin	
LF4	CXR		
LF5	Chest CT		
Part four: Disease severity and outcome			
DO1	Severity	Mild	
		Moderate	
		Sever	
		Critical	
DO2	Outcome	Survivor (discharged)	
		Death	
Part five: Treatment, intervention and complication			
TX1	Medication given during hospital Stay	Antibiotics 1.Ceftriaxone 2.Azithromycin 3.Cefepime 4.Meropenium 5.Ceftazidime 6.Meteronidazole 7.Other	
		Steroid 1.Yes 2. NO	
		Vasopressor 1.Yes 2. NO	
		Anticoagulant 1.Yes 2. NO	

TX2	Intervention done	Oxygen therapy 1.Yes 2. NO	
		RRT 1.Yes 2. NO	
		MV 1.Yes 2. NO	
		Other	
TX3	Complication	No complication	
		ARDS, AKI, HAI, Sepsis and Coagulopathy	
		ARDS, AKI, HAI, Sepsis	
		Coagulopathy: PE, PTE, DVT	
		Acute cardiac injury MI	

11.2. Assurance of Principal Investigator

I the undersigned agree to accept all responsibilities for the scientific and ethical conduct of the research project. I will provide timely progress report to my advisor and seek the necessary advice and approval from my primary advisors in the course of the research. I will communicate timely to my advisors all stakeholders involved in the study including any source of funding for this research.

Name of the student: _____

Signature: _____

Date: _____

Approval of the Primary Advisor

Name of the primary advisor: _____

Signature: _____

Date: _____

Approval of the Co-Advisor

Name of the primary advisor: _____

Signature: _____

Date: _____