

Original Research Article

Study Of Potential Utility Of Predictive Laboratory Parameters For Early Triage Of SARI Patients

ABSTRACT

PURPOSE: The novel corona virus (COVID-19), which began in Wuhan, China, in December 2019, has been declared pandemic by the World Health Organization (WHO) on 11th March 2021. Till now, even when nations are in race for developing vaccine against COVID-19, diagnosing the cases and isolating them still remain the only way to contain the disease spread. Hence, effective triage of the suspected COVID 19 patients, becomes an effective tool in preventing disease spread, especially in developing nations.

PATIENTS & METHODS: Retrospective case control study conducted over a period of one month including 286 consecutive SARI patients admitted in a tertiary care hospital in Delhi. The patients were divided into COVID-19 positive and COVID-19 negative groups based on their results of reverse transcriptase-polymerase chain reaction (RT-PCR) for COVID-19. The demographic data, and routine hematological and biochemical parameters including derived parameters like Neutrophil lymphocyte ratio (NLR), derived NLR ratio, Platelet lymphocyte ratio (PLR) and Prognostic nutrition index (PNI) were analyzed and compared between the two groups using SPSS version 21.0. A p value of <0.05 was considered significant.

RESULTS: The COVID-19 positive patients had a significant age difference, a significantly higher lymphocyte percentage and SGPT, and a significantly lower Absolute Neutrophil Count (ANC), NLR and platelet count. No significant difference in other haematological and biochemical parameters was found.

CONCLUSION: NLR, platelet count, lymphocyte percentage and SGPT are the simple laboratory biomarkers of inflammation that can be utilized for screening and triage in COVID 19 disease with variable sensitivity and specificity.

KEYWORDS: Age, COVID-19, lymphocyte percentage, NLR, platelet count, routine, SGPT, triage

INTRODUCTION:

The novel corona virus (COVID-19), which began in Wuhan, China, in December 2019, has been declared pandemic by the World Health Organization (WHO) on 11th March 2021.¹ Globally it has resulted in 120,667,101 confirmed cases including 2,670,274 deaths² and has threatened global public health. North America, South America, Europe and Asia are the most affected (maximum number of cases) continents by this pandemic so far. India itself has reported 11,474,605 confirmed cases.² In spite of rapid development of knowledge, precautionary measures, and clinical trials, regulatory bodies and government administrations are facing a great challenge globally in various aspects to contain the spread of the disease. Development of vaccine has been a global challenge and its worldwide administration is still another one. Hence, still at present, a key to slow the disease

spread is rapid diagnosis and isolation of cases and this relies on the effective triage protocols for identifying suspected COVID-19 patients and their isolation or diagnosis for a timely management and optimal utilization of the limited human and technical resources for testing and management of COVID-19 disease.³ Currently, the diagnosis relies on nucleic acid detection. However because of a relative shortage of detection kits and false negative results due to low viral load, many patients are left undetected and undiagnosed.

Due to similarity in the clinical presentation of COVID 19 and COVID-19 like respiratory illnesses, many true COVID-19 patients were left undiagnosed at fever clinics causing cross infection, or were self isolated at home, which was however often ineffective and could lead to family cluster infections and poor prognosis because of delayed treatment.

The clinical characteristics of COVID-19 have been elaborated, however an outline of the most representative laboratory abnormalities found in patients with COVID-19 infection is still lacking. Laboratory diagnostics play an important role in etiological diagnosis, epidemiologic surveillance and assessing disease severity, for defining the prognosis, for following-up patients, for guiding treatment and for their therapeutic monitoring.⁴ In India, the initial COVID-19 testing strategy included symptomatic patients who had a history of international travel, symptomatic contacts of laboratory-confirmed COVID-19 patients and symptomatic healthcare workers managing Influenza like illness (ILI)/severe acute respiratory illness (SARI) patients.⁵ However, about one third of COVID-19 positive SARI cases did not have

any history of contact with a laboratory-confirmed case or international travel history.⁶

Therefore, the aim of this article is to provide a brief overview on the hematological and biochemical laboratory parameters of systemic inflammation and immune status in COVID-19 positive patients and compare them with COVID-19 negative severe acute respiratory illness (SARI) patients to assess their potential utility as soft pointers for COVID-19 infection in resource constraint settings.

MATERIALS & METHODS:

Study Design: It is a retrospective case control study conducted over a period of one month (September 5, 2020 to October 4, 2020) including 286 consecutive SARI patients admitted in ABVIMS & Dr Ram Manohar Lohia hospital, a designated nodal centre for management of COVID-19 patients in New Delhi. The study protocol was approved by the Ethics Committee of ABVIMS & Dr Ram Manohar Lohia hospital.

(ethical code: 403(52/2020)IEC/ABVIMS/RMLH136)

The study included all adult patients who were diagnosed as SARI at our fever clinics and at SARI casualty as per WHO case definition⁷ and screened for COVID-19. The patients were divided into cases and control groups based on their results of real time reverse transcriptase-polymerase chain reaction (RT-PCR) for COVID-19, i.e. COVID-19 positive (cases) and COVID-19 negative (control group). The

information recorded included the demographic data, hematological and biochemical parameters of inflammation including Complete Blood Counts (CBC), Renal function tests, Liver function tests, Total protein, and Serum albumin. Neutrophil lymphocyte ratio (NLR), derived NLR ratio, Platelet lymphocyte ratio (PLR) and Prognostic nutrition index (PNI), were calculated as per the case definitions described. All the data was compared between the COVID positive and COVID-19 negative groups. Also the accuracy of significant laboratory parameters in predicting cases with positive RT-PCR was evaluated using area under the ROC curve (AUC). The optimal cut off values, sensitivity and specificity of the statistically significant parameters were also calculated after the analysis of ROC curve.

Definitions: NLR is the ratio of absolute neutrophil count to absolute lymphocyte count. dNLR is the ratio of neutrophil count to WBC count minus neutrophil count (ANC/TLC-ANC). PLR is the ratio of platelet count to absolute lymphocyte count. PNI (Prognostic Nutrition Index) is a common inflammatory biomarker calculated as $(10 \times \text{serum albumin g/dL} + 0.005 \times \text{total lymphocyte count})$.

Statistical analysis: The data was entered in MS EXCEL spreadsheet and analysis was done using Statistical Package for Social Sciences (SPSS) version 21.0. Categorical variables were presented in number and percentage (%) and continuous variables were presented as mean \pm SD and median. Normality of data was tested by Kolmogorov-Smirnov test. If the normality was rejected then non parametric test was used. Quantitative variables were compared using Mann-Whitney Test (as the data sets were not normally distributed) between the two groups. Qualitative variables were compared using Chi-Square test/Fisher Exact test. Receiver operating

characteristic curve was used to find out cut off point of parameters for predicting positive findings. A p value of <0.05 was considered statistically significant.

RESULTS:

Demographic Characteristics: Two hundred eighty six SARI cases were included in the study. The result of RT-PCR for COVID-19 was positive in 127 (44.4%) cases and negative in 159 (55.5%). The cases ranged from 15 to 85 years in age with a mean age of 50.76 ± 15.77 years for positive group and 46.47 ± 18.92 years for negative group. There was a significant age difference between the two with COVID-19 positivity being more frequent in patients >50 years ($p=0.045$). (Figure 1) Groups of cases with positive and negative RT-PCR showed no significant gender preference ($p=0.13$). (Figure 2)

Blood Routine Parameters:

Table I compares the laboratory parameters of COVID-19 positive patients with the negative group. The COVID-19 positive patients had a significantly higher lymphocyte percentage (19.7% vs 16.7%, p value <0.05) and SGPT (74.21 vs 42.21 U/L, p value <0.05), and a significantly lower Absolute Neutrophil

Count(ANC)[7255.4 vs 8894.1/ μ l], a significantly reduced NLR(5.74 vs 6.98, p value<0.05) and platelet count (1.8 vs 2.16 lacs/ μ l]. Though not statistically significant, but we also found that the mean total leukocyte count was lower in COVID positive patients as compared to the control group (9417 vs 11108/ μ l] but not reaching the cutoffs for leucopenia. Also the PLR was lower in COVID positive patients (155.8 vs 183.3) but with a p value of 0.091, hence not statistically significant.

No significant difference in other hematological and biochemical parameters was found between the patient and the control group.

Receiver operating curves (ROC) curves were drawn for the laboratory parameters that showed a significant p value. Taking the null hypothesis into account as true area = 0.5, the parameters that showed value in predicting cases with positive RT-PCR for COVID-19 were age (AUC=0.544), lymphocyte count (AUC=0.588), NLR (AUC=0.579), platelet count (AUC=0.598) and SGPT (AUC=0.602). (Figure3, table II). The ANC could not be used as a potential diagnostic marker for subsequent analysis because its AUC was less than 0.50.

Appropriate cut off values for the significant parameters (Age, lymphocyte count, NLR, platelet count & SGPT) were statistically derived using ROC curves.(Table III). The cut off values were 15.5% for lymphocyte percentage (sensitivity=70.2% and specificity=58%), 1.74 lacs/ μ l for platelet count (sensitivity=64.2% and specificity=55.9%), 29.5U/L for SGPT (sensitivity=70.2% and specificity=55.9%),

and 3.8 for NLR (sensitivity=62.9% and specificity=50.4%). Lymphocyte percentage and SGPT had the highest sensitivity while SGPT had the highest specificity in diagnosing COVID 19 positive patients.

DISCUSSION:

Considering the rapid spread and the global health impact of COVID 19, it is required to continue research on new triage and diagnostic modalities to improve case identification. Molecular identification of SARS-CoV-2 in low-resource settings remains centralized and time-consuming, resulting in a delayed diagnosis of COVID-19. The dysregulation of immune response and excessive inflammation are the key elements of pathogenesis of COVID-19.⁷ The blood biomarkers of systemic inflammation reflect the immune status and hence are potential indicators for prognosis and diagnosis of COVID-19 patients. This study aimed to identify frequently performed laboratory parameters that can assist in COVID-19 case identification. The study revealed a significantly raised lymphocyte percentage and SGPT levels, and a significantly lower ANC, NLR and platelet count in COVID 19 positive patients. All the other hematological and biochemical parameters did not show any role in predicting COVID-19 positive cases.

Various studies have found similar results in severe COVID-19 patients. A meta-analysis by including three studies in patients with severe or fatal COVID 19 found an increase in leucocyte and neutrophil count and a decrease in lymphocyte and platelet count.⁸ However relative to non-COVID-19 infections, significantly lower

absolute leucocyte or neutrophil and platelet counts during early stages of the disease have been demonstrated in a meta analysis of seven studies although the mean leucocyte count of the studies included did not exceed the lower limit for categorization as leucopenia or neutropenia. They found that with COVID-19 disease progression, both leukocyte and neutrophil counts were significantly higher in the severe groups.⁹ We too have found a lower mean leucocyte count in the COVID-19 cohort and a significantly lower ANC and NLR. Study by Huang et al revealed that asymptomatic COVID-19 patients with similar viral load as symptomatic patients, had a higher lymphocyte percentage indicative of a better immune response against the virus. This can also possibly explain a higher lymphocyte percentage during early course of the disease.¹⁰

However, many studies found neutrophilia i.e. raised ANC and NLR in COVID-19 patients. It was initially thought to be due to secondary bacterial infections but recent studies have implicated excessive neutrophilia and neutrophil extracellular traps (NET) as the cause of hyper inflammation and lung injury.¹¹ More so the exacerbated NET formation is thought to drive a cascade of inflammatory reactions that favors micro thrombosis, thus suggesting raised ANC and NLR to be independent prognostic features as well as predictors of severe COVID-19 infection.¹¹ But, there are studies which have found results similar to our results. They found a significantly decreased NLR in COVID positive vs negative patients. They observed an AUC of 0.739, cut off =1.8, sensitivity of 0.59 and specificity of 0.76, and proposed the use of NLR as an early diagnostic marker for COVID 19.¹² Yang et al. and Qu et al. in their respective studies had elaborated upon the predictive role of raised NLR as

circulating biomarker that can represent viral hyper inflammation and immune status in severe COVID-19 and described it as an independent biomarker for poor prognosis.^{13,14} This study though caters only Chinese population with severe COVID - 19 disease, hence may not be representative of a heterogeneous population with mild, moderate and severe patients. Also studies have found a decreased mean ANC 3.89(2.61-6.33) and mean NLR of 3.21(1.94-7.11) in all the COVID positive patients vs a mean ANC and NLR of 7.41 and 12.41 respectively in severe COVID-19 patients (non- survivors).¹⁵ Another study found a normal or mildly reduced NLR and ANC <1.2 and <4000/ μ l respectively in asymptomatic to mild cases of Covid-19. Severe cases were coupled with leucocytosis and increased NLR >3.13 or higher than >5.0.¹⁶ Our study included a heterogeneous population of patients ranging from mild to severe category of COVID-19 which may explain our results.

Thrombocytopenia has been the most common hematological change seen in patients with COVID-19 in various studies including ours. Xu P et al. proposed direct bone marrow suppression along with immune mediated destruction of platelets and platelet aggregation in lungs to be the three mechanisms responsible for thrombocytopenia in COVID-19.¹⁷ The immune dysfunction and consequent cytokine storm associated with COVID-19 infection leads to lung injury and destruction of hematopoietic destruction and bone marrow growth inhibition. The damaged lung tissues and pulmonary endothelial cells may activate platelets in the lungs, resulting in platelet aggregation and formation of micro thrombi. The COVID-19 infection may also increase the levels of auto antibodies and immune complex formation which gets

deposited on the surface of platelets, and subsequent recognition and destruction by the reticuloendothelial system.

It can be noted that the ACE-2 protein has an abundant expression in intestinal epithelial cells, renal tubular epithelial cells, alveolar epithelial cells, heart, artery smooth muscle cells and gastrointestinal cells. Therefore apart from lungs, COVID-19 is also speculated to infect liver, gastrointestinal system and kidney which may lead to abnormal liver functions, diarrhea and acute kidney injury.¹⁸ A meta-analysis of 521 studies found that patients with COVID-19 had higher than expected prevalence of liver injury, and the extent of the injury was associated with the severity of the disease. They found a substantial elevation in enzymes (>3 times) in almost 16% of the COVID-19 positive patients.¹⁹ We found a significant elevation in SGPT levels of COVID-19 patients however SGOT, ALP and albumin did not show any significant difference. On the contrary, Chen et al., found that albumin had decreased, but SGPT and SGOT showed no significant changes.²⁰ Furthermore another meta-analysis of 128 studies by Kumar et al. found hypoalbuminemia [61.27% (48.24–72.87)] and elevations of SGPT [23.28% (19.92–27.01)], and SGOT [23.41% (18.84–28.70)] to be the most frequent abnormalities.²¹ The liver enzyme derangement can be due to direct viral injury, multiple antibiotics and antiviral drugs intake prior to presentation or due to the COVID-19 infection induced cytokine storm.

We found no significant differences or derangements in the renal function tests i.e. blood urea, serum creatinine and uric acid in COVID-19 positive and negative SARI patients. Sharma et al found similar findings of essentially normal renal profile in

COVID-19 patients.²² Nevertheless a close monitoring of the renal function in COVID-19 patients is required.

We made an attempt to study the impact of overall nutritional status as a marker of susceptibility to COVID-19 by comparing PNI between COVID-19 positive and negative SARI patients. No significant difference was found between the PNI of the two groups. However study by Wang et al found a significant difference in PNI between COVID-19 survivors and non survivors, and suggested it as a predictive biomarker for severity and progression in COVID-19 patients.¹⁵ However no study done so far has utilized its utility as diagnostic marker for the purpose of triage of COVID-19 patients. Therefore we suggest that the nutritional status has no bearing on the susceptibility to COVID-19 infection, however it may have an impact on the prognosis of the disease.

The core strength of the present study is the inclusion of a large, contemporary, COVID-19 negative control population, which allowed us to critically reappraise the haematological features of patients with COVID-19.

There were some limitations in our study. First, it was a retrospective study and the sample size was small. Secondly, the lack of clinical data is one of the deficiencies of this study, so we can't rule out the influence of stress response and treatment on blood routine parameters. Further research is needed to overcome these limitations.

Nevertheless, our results provided important illumination on this topic.

CONCLUSION: NLR, platelet count, lymphocyte percentage and SGPT are the simple laboratory biomarkers of inflammation that can be utilized for screening and triage in COVID-19 disease with variable sensitivity and specificity. Yet further studies with larger sample size are required to better validate the results.

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Table 1: Comparison Of The Laboratory Parameters Between The Cases With Positive And Negative RT-PCR For COVID-19 Infection

Parameter (Units; mean±SD)	COVID-19 RT-PCR		p value
	Negative (N=159)	Positive (N=127)	
Hemoglobin(gm/dL)	11.32±2.10	11.30±2.53	0.935
Total leucocyte count(/ μ l)	11108.00± 6618.24	9417.30± 8357.54	0.057
Neutrophil count(%)	77.48 ±11.12	75.14±11.79	0.087
Lymphocyte count(%)	16.74 ±9.82	19.73±11.25	0.017
Absolute neutrophil count (ANC)(/ μ l)	8894.10 ± 5824.97	7255.40± 6306.43	0.023
Absolute lymphocyte count (ALC)(/ μ l)	1616.70 ± 1078.90	1672.00± 1961.10	0.763
Neutrophil lymphocyte ratio(NLR)	6.98 ±5.24	5.74 ±6.98	0.033
Derived NLR (dNLR)	4.70±3.12	4.20±3.05	0.17
Platelet count(/ μ l)	216060± 92773.17	180980± 80426.78	0.001

Platelet lymphocyte ratio (PLR)	183.30 ±131.69	155.88±140.67	0.091
Urea(mg/dL)	59.57±74.26	52.45±53.03	0.364
Serum creatinine(mg/dL)	1.75±2.61	1.71±2.59	0.884
Uric acid(mg/dL)	7.02±5.61	6.64±3.38	0.497
Total bilirubin(mg/dL)	1.23±3.60	0.74±0.63	0.127
Direct bilirubin(mg/dL)	0.42±0.87	0.36±0.73	0.513
Indirect bilirubin(mg/dL)	0.81±3.15	0.45±0.41	0.197
SGOT*(U/L)	54.52±51.99	62.64±72.86	0.276
SGPT**(U/L)	42.21±45.58	74.21±179.45	0.033
ALP***(U/L)	123.61±84.8	126.33±127.24	0.83
Total protein(gm/dL)	11.58±56.75	6.59±0.78	0.335
Serum albumin(gm/dL)	3.19±1.1	3.21±0.61	0.903
PNI****	37.79±14.51	39.40±13.99	0.344

Notes:

Abbreviations: *SGOT,Serum glutamic oxaloacetic transaminase **SGPT,Serum glutamic pyruvic transaminase ***ALP,Alkaline phosphatase ****PNI,Prognostic nutrition index

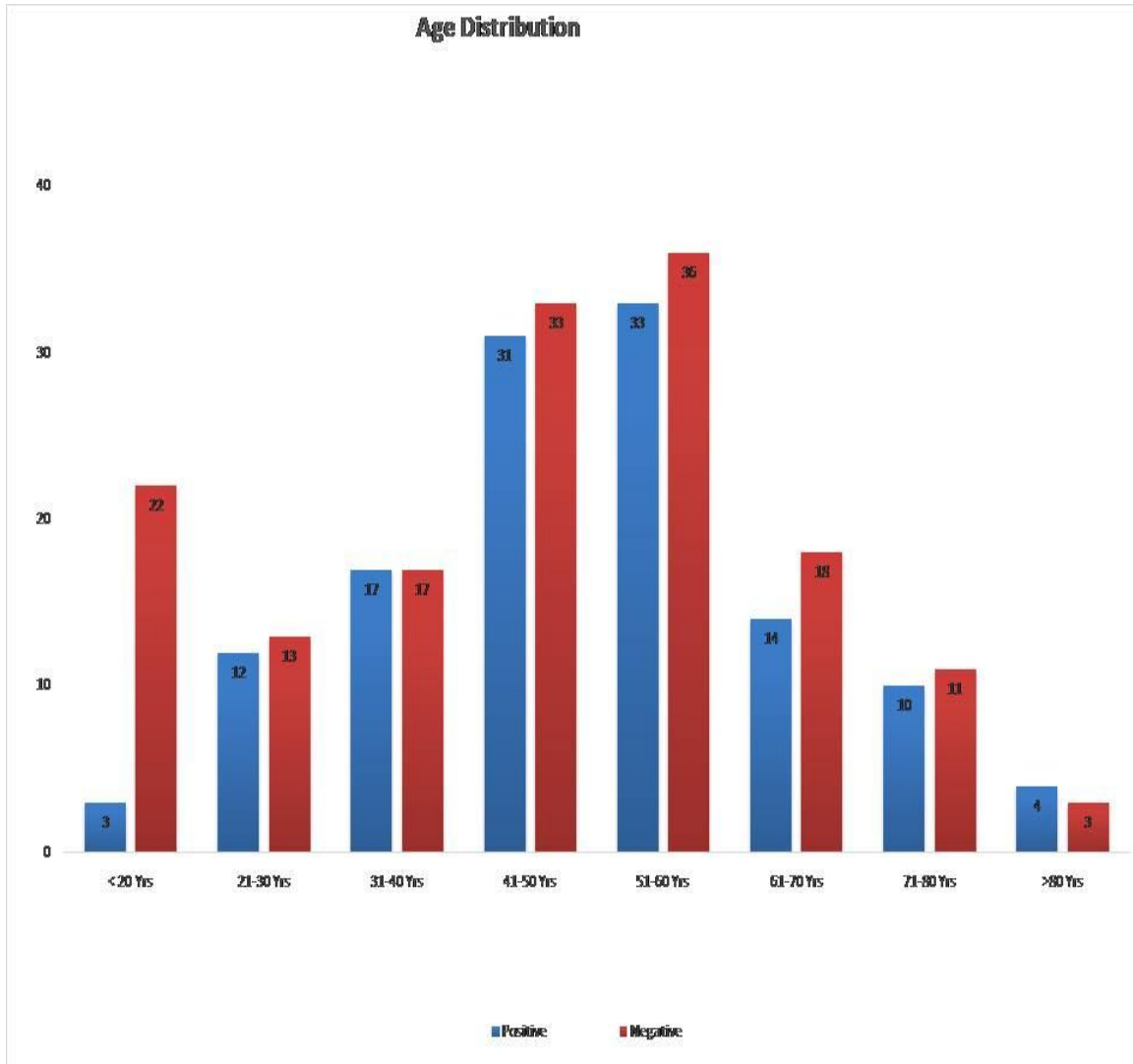
Table 2: Area Under The Curve Of Significant Parameters

Area Under the curve					
Test Result Variable(s)	Area under the curve(AUC)	Standard error	Asymptotic Sig ^b	Asymptotic 95% Confidence interval	
				Lower Bound	Upper Bound
Age	0.544	0.035		0.46	0.612
LYMPHOCYTE COUNT (%)	0.588	0.035	70.2%	0.52	0.656
NLR	0.579	0.034	62.9%	0.513	0.646
PLATELET COUNT (/μl)	0.598	0.034	64.2%	0.532	0.664
SGPT (U/L)	0.602	0.034	70.2%	0.534	0.669
<p>The test variables has at least one tie between the positive actual state group and the negative state group. Statistics may be biased.</p> <p>a. Under the non parametric assumption</p> <p>b. Null hypothesis : true area=0.5</p>					

Table 3: Diagnostic Performance Of Laboratory Parameters In Differentiating COVID-19 Positive Cases From Controls

Test Result Variable(s)	Positive if Greater Than or Equal To	Sensitivity	Specificity
LYMPHOCYTE COUNT (%)	12.5-15.5	70.2%	58%
NLR	3.895-4.325	62.9%	50.4%
PLATELET COUNT (/ μ l)	174000-188000	64.2%	55.9%
SGPT (U/L)	24.5-29.5	70.2%	59.4%

Figure 1: Age Distribution Of SARI Patients.



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Figure 2 : Gender Distribution Of SARI Patients.

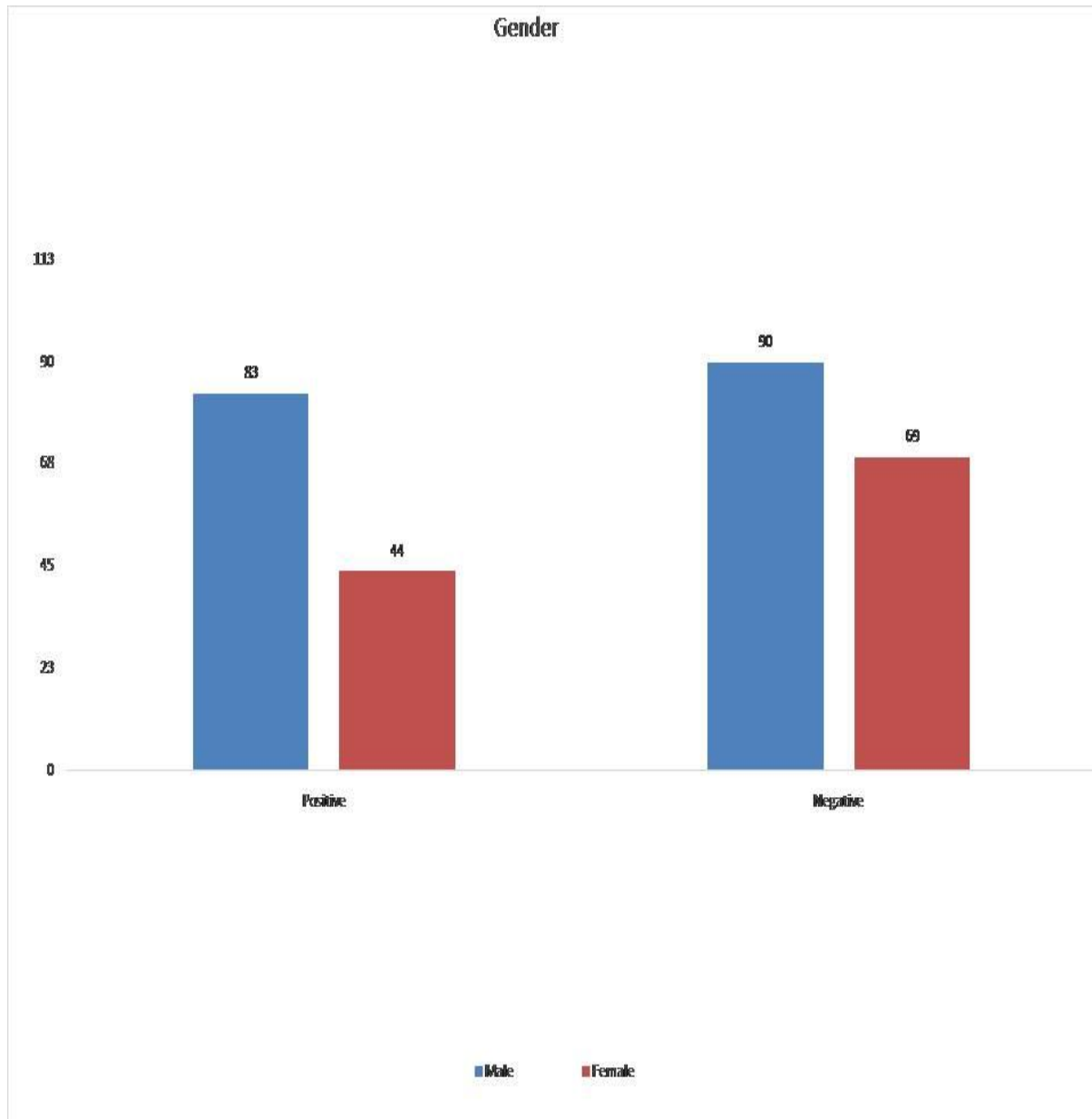
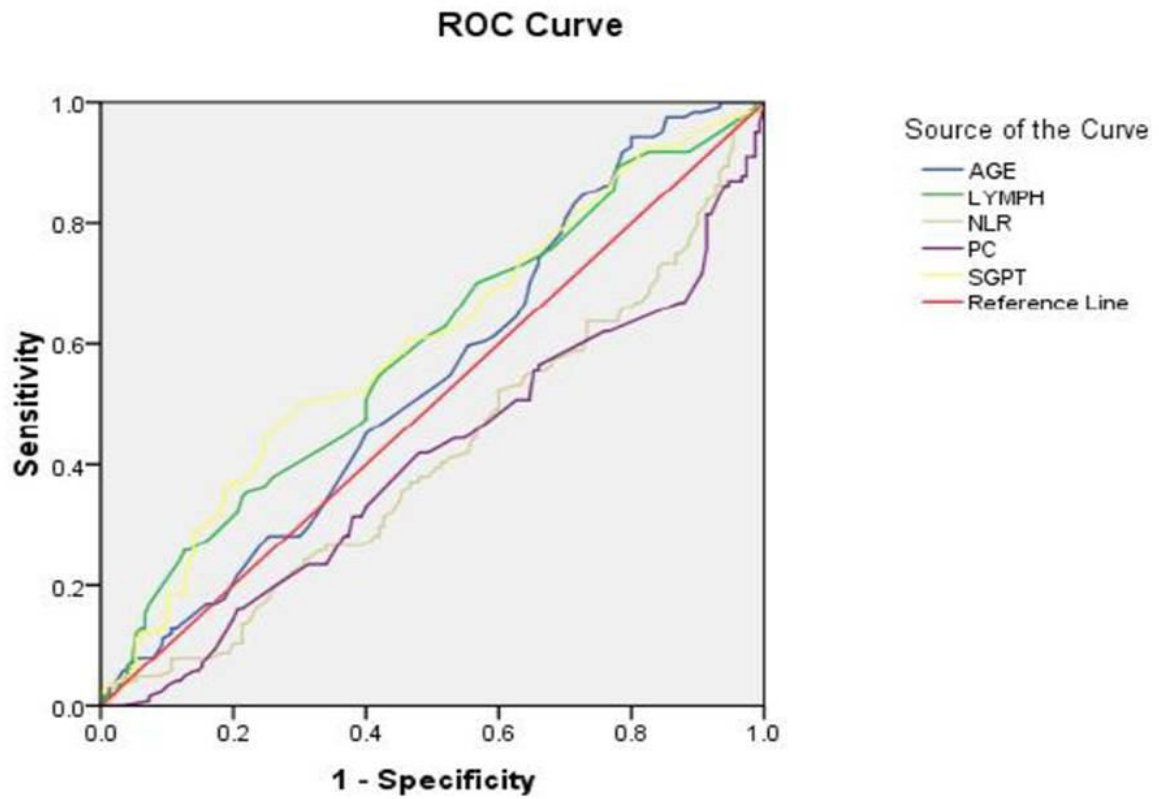


Figure 3: Area Under The Receiver Operating Characteristic Curve Of Different Laboratory Parameters In Predicting Cases With Positive RT-PCR For COVID-19.



Diagonal segments are produced by ties.

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