

Original Research Article

Diagnostic Laboratory Markers of COVID-19 Positive Patients in a Tertiary Care Hospital of Southern Rajasthan**Priyanka Purohit^{1*}, Neelam Jain², Swati Jindal³, N.Mogra⁴, M.L.Gupta⁵, G.Mukhiya⁶**¹*P.G. Resident, Department of Pathology, GMCH Udaipur, Rajasthan, India*²*Assistant Professor, Department of Pathology, GMCH Udaipur, Rajasthan, India*³*Assistant Professor, Department of Pathology, GMCH Udaipur, Rajasthan, India*⁴*Dean GMCH, Udaipur, Rajasthan, India*⁵*H.O.D., Department of Pathology, GMCH Udaipur, Rajasthan, India*⁶*Professor, Department of Pathology, GMCH Udaipur, Rajasthan, India***Received: 05-04-2021 / Revised: 20-05-2021 / Accepted: 19-06-2021****Abstract**

Introduction: As Coronavirus disease is spreading, there is urgent need of identification of clinical and laboratory predictors for progression towards severe and fatal forms of this illness. Corona virus infected people have mild to moderate symptoms. However, 15-32% develops severe symptoms with a case fatality rate of 1-15%. Covid-19 is a systemic infection with adverse impact on hematopoietic system and hemostasis.

Aim: The main aim of this study was to predict common laboratory parameters which are altered in covid-19 positive patients. **Methods:** A retrospective study was conducted at Geetanjali Medical College and Hospital for 2 months during first wave of Covid-19. The confirmed 101 positive patient's list was collected from Microbiology DPT. HIS system of GMCH Udaipur. This study includes all common laboratory parameters of Covid-19 diagnosed patients. Laboratory parameters included in this study were – Complete blood count, CRP, D-dimer, A/G ratio, S.Ferritin, S.Creatinine and LDH. The data was collected using HIS system. **Conclusion:** This study predicted that NLR and S.Ferritin get deranged in majority and were best laboratory markers to look after during COVID period. They should be given ICU care. The next good predictors were CRP, S.Creatinine and D-dimer. The tests of LDH and A: G ratio should be avoided as they are poor tests. This has been decided on the basis of ROC curve.

Keywords: ICU, D-dimer, Ferritin, CBC, LDH, NLR, S.Creatinine.

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Introduction

Coronavirus disease also known as novel corona virus has emerged as a global pandemic; first occurred in Wuhan, Hubei province, China in early December 2019 [1]. Till 22 October 2020, 41,104,946 confirmed cases and 1,128,325 deaths in world have been reported to WHO. [2] SARS-CoV-2 belongs to the Sarbecovirus subgenus of Coronaviridae family.

This is an enveloped virus having single-stranded positive sense RNA viral genome. Virions are spherical, with the envelope having spiked glycoprotein inserted in it [3]. After its entry into lungs, SARS-CoV-2 stimulates the activity of immune cells, increases cytokine production, and activates other pathogen resistance mechanisms. Initially there is fast and robust virus replication and later on IFN generation, which results in disproportionate inflammatory host responses provoking grave lung alterations [4, 5]. Although it is well documented that the primary manifestation of COVID-19 is respiratory tract infection, it is regarded as a systemic disease which affects multiple systems, including cardiovascular, respiratory, gastrointestinal, neurological, hematopoietic and immune system. [6]

**Correspondence*

Dr. Priyanka Purohit

P.G. Resident, Department of Pathology, GMCH, Udaipur, India

E-mail: priyanka.purohit5@gmail.com

Severe COVID-19 can lead to critical illness, with acute respiratory distress syndrome (ARDS) and multi-organ failure (MOF) as its primary complications, eventually followed by intravascular coagulopathy [7]. COVID-19 patients are categorized as mild, moderate, severe, and critical types on the basis of clinical symptoms and laboratory test results. Most of the patients (81%) fall into mild/moderate category. Severe and critical ones who need hospitalization comprise only 14% and 5% of infected cases, respectively.

Intensive care unit (ICU) is needed in around 20% of hospitalized patients. Mortality rate of ICU admitted COVID-19 patients is reported quite high around (61.5%) (8). Basic pathology behind the COVID-19 pneumonia is the cytokine storm produced by the inflammation induced by the virus. [9].

Severe inflammatory responses denote to weak adaptive immune response, and hence there is imbalance in immune response. Therefore inflammatory parameters are potential predictors for the prognosis of COVID-19 patients, it include circulating biomarkers that can represent inflammation and immune status [10]. There is direct correlation of markers of hyperinflammatory state and severity of covid 19 patients. Apart from the clinical symptoms and pulmonary computed tomography (CT) findings, various laboratory fluctuations include complete blood count (CBC) variables, cardiac and coagulation parameters, renal and liver function tests, and inflammation-related factors in COVID-19 confirmed patients. [11, 12] Various hemocytometric markers such as peripheral white blood cell

(WBC) count, neutrophil (NEU)-to-lymphocyte (LYM) ratio (NLR), derived NLR ratio (d-NLR, neutrophil count divided by the result of WBC Count minus neutrophil count), platelet-to-lymphocyte ratio (PLR) and lymphocyte-to-monocyte ratio (LMR) are proven to be indicators of the systematic inflammatory response [10] and hence they can be used as predictors for the prognosis of patients with Covid 19 pneumonia. [13] Various biochemical parameters such as C-reactive protein (CRP), D-dimer, albumin, ferritin and LDH levels also correlate with disease severity [14]. Our aim of study was to analyse various laboratory parameters which were helpful in depicting severity and hence prognosis of Covid 19 patients, so that patients can be stratified as severe or non severe. This will help in early initiation of treatment and supportive care in patients so as to decrease mortality.

Methods

This is a retrospective cross sectional study, in which data from one hundred and one patients (101) with confirmed Covid-19 reports were collected. These patients were treated in a Tertiary care center of Southern Rajasthan from June 2020 to July 2020 and were analyzed. Only confirmed cases by an accredited laboratory utilizing real time reverse transcriptase polymerase chain reaction assay (RT-PCR) were included. Data of Laboratory parameters including – Complete blood count, S.Ferritin, CRP, D-dimer, S.Creatinine, A/G ratio, S. LDH were collected by Central laboratory HIS System. As we are not disclosing any patient's identity, therefore ethical clearance not needed. We had divided our patients among ICU and Non ICU categories on criteria of their SPO₂ levels. Patients having <90 SPO₂ were put in ICU patients category and rest in Non ICU.

Statistical Analysis

Descriptive statistics includes means and standard deviation which describes laboratory outcome. ROC curve and AUC curve were used to analyze the optimal cut off for predicting ICU patients. AUC 0.9 to 1 was defined as very good, 0.7 to 0.8 as good, 0.6 to 0.7 as sufficient, 0.5 to 0.6 as bad, and <0.5 as poor (useless test). A p value <0.5 denoted statistical significance. t-pair test was used for comparison of ICU and Non ICU patients and p value was calculated.

Outcome of Laboratory Parameters

We studied about total 101 patients, among which 78 were males and 23 were female. We divided them in 3 groups according to age, <20 yrs., 20-50yrs., >50yrs. We found only 1 patient beyond 20 yrs., 65 were 20-50 yrs. and 35 were >50 yrs.

The biochemical laboratory findings in Table 1 indicates that the S.Ferritin level for Non-ICU patients (647.41±590.39) was lower compared to the ICU patients (M=270.56±353.66). The reported difference was statistically significant (p=0.001). Table 1 indicates that D-dimer and CRP were statistically significantly different among ICU (M=99.04±9.95) and Non ICU patients (1.092±2.21) and CRP (4.807±15.95), Non ICU (27.426±63.506). However A: G ratio and S.Creatinine values does not vary between ICU and Non ICU Covid positive patients.

The CBC findings indicates that the neutrophils to lymphocytes ratio was significantly higher among ICU patients (7.62±4.93) compared to Non ICU patients (2.199±1.69).

Table 1: Laboratory parameters among the ICU and Non ICU Covid 19 positive patients

S. No.	Variables	ICU(n=26) Mean±(SD)	Non ICU(n=75) Mean±(SD)	P-value
1.	NLR	7.602(4.93)	2.199(1.691)	<0.0001
2.	PLR	271.48(193.95)	146.65(84.79)	<0.0001
3.	D-dimer	99.04(9.95)	1.092(2.21)	<0.0001
4.	A:G ratio	1.328(0.727)	1.425(0.803)	0.4346
5.	S.Creatinine	4.807(15.95)	1.386(2.421)	0.0792
6.	S.Ferritin	647.41(590.39)	270.56(353.66)	0.0014
7.	LDH	407.40(305.72)	283.44(109.67)	0.0080
8.	CRP	107.22(130.41)	27.426(63.507)	0.0010

Discussion

This study depicted the difference in various biochemical and CBC parameters between ICU and Non ICU patients. The results showed increased values of S.Ferritin, CRP, D-dimer, LDH and NLR among ICU patients compared to Non ICU patients.

Figure 1, 2 shows the area under ROC curve of the assessed biochemical parameters and CBC parameters. The biochemistry with very good accuracy was NLR and S.Ferritin, with good accuracy were D-dimer, S.Creatinine and CRP.

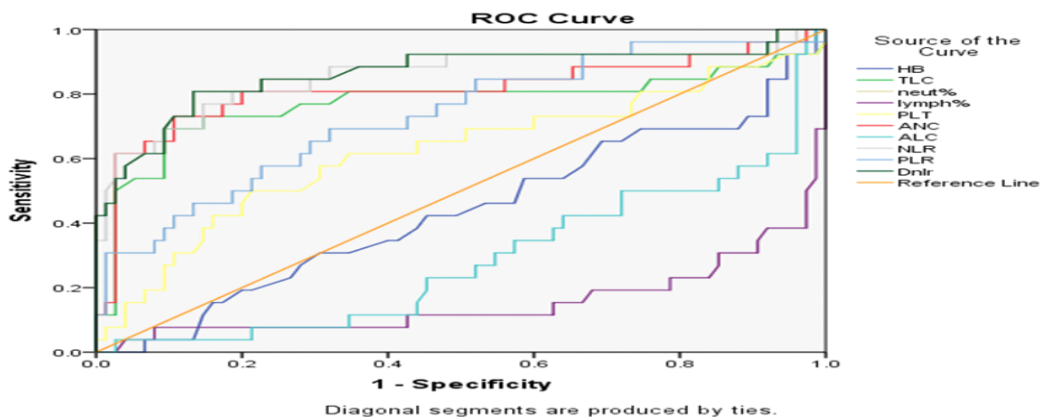


Fig 1: ROC curve

Table 2: AUC (Area under curve)

Test Result Variable(s)	Area under curve	P value
HB	.436	.330
TLC	.771	.000
neut%	.863	.000
lymph%	.154	.000
PLT	.621	.067
ANC	.812	.000
ALC	.286	.001
NLR	.855	.000
PLR	.731	.000

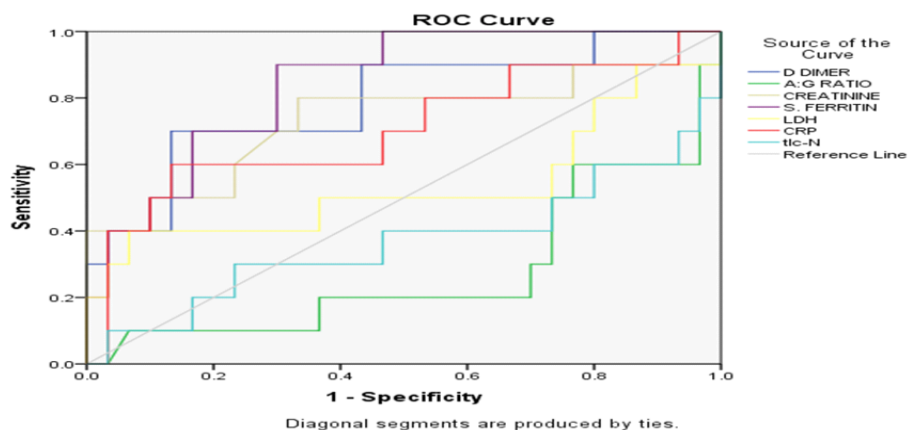


Fig 2: ROC curve

Table 2: AUC (Area under curve)

Test Result Variable(s)	Area under curve	P value
D DIMER	.790	.007
A:G Ratio	.275	.035
Creatinine	.730	.031
S. Ferritin	.843	.001
LDH	.537	.731
CRP	.703	.057

Figure-4(Table-3) The area under the ROC curve (AUC) results for lab. parameters among the ICU Covid patients, were considered excellent for AUC values between 0.9-1, good for AUC values between 0.8-0.9, fair for AUC values between 0.7-0.8, poor for AUC values between 0.6-0.7 and failed for AUC values between 0.5-0.6

The findings in Table 2,3 indicates that statistically significant ROC curves were interpreted based on criteria where AUC where AUC 0.9 to 1 was defined as excellent accuracy, 0.8 to 0.9 as very good, 0.7 to 0.8 as good, 0.6 to 0.7 As sufficient, 0.5 to 0.6 as bad, and less than 0.5 as poor (useless test). As shown in Table 3 neutrophils, AST and LDH's ROC curves are good tests while CRP curve is a very good test. However, lymphocyte curve is a poor test. As shown in table 2 statistically significant AUCs were obtained for S.Ferritin (AUC=0.790,p=0.007), NLR (AUC=0.855,p=0), D-dimer (AUC=0.790,p=0.007), CRP(AUC=0.703,p= 0.057) and creatinine (AUC=0.730 p=0.31). LDH and A: G ratios were bad tests.

It is also noted that S.Ferritin, CRP, D-dimer, NLR have good specificity and sensitivity in prediction of ICU patients. Henry et al states that patients with respiratory distress WBC count, lymphocyte count, platelet count, S. ferritin are markers for potential progression to critical illness [19]. Zu Li Chang depicts that Covid positive patient's hematological parameters except D-dimer concentration, are not associated with an increased risk of death. Dynamic measurements of platelets, fibrinogen, CRP, and LDH correlate with

risk of death [20]. Terpos et al concluded that COVID-19 is associated with hypercoagulability. Preventive measures for thromboprophylaxis and early identification of lethal complications including DIC in order to improve patient outcomes [21]. Ai-Ping Yang et al concluded that elevated NLR can be considered independent biomarkers for poor clinical outcomes [21]. Increased NLR ultimately depicts lymphopenia. Wang et al. [16] tells the importance of lymphopenia in the ICU Covid 19 patients.

The biochemical test findings of this study, showed elevated level of CRP, S.Ferritin, D-dimer, NLR among the ICU patients supports the conclusion made by Chavin et al [18]. However, non significant values of this LDH contradicts the previous researches made by Chavin et al [18] and Mardani et al [17], which explains increase level of LDH in ICU Covid patients. Various researchers have also noted that severe cases of Covid-19, develop neutrophilia during hospitalization [15, 16]. It is also noted that S.Ferritin, CRP, D-dimer, NLR have good specificity and sensitivity in prediction of ICU patients. The reported excellent accuracy of NLR in the patient of Covid 19 supports the findings obtained by Mardani et al [17]. The findings of this study is significant in early treatment and identification of positive patients in need of ICU care by giving them ICU admissions and vigorous treatment and in case if ICU beds are not available then the ICU injectables can be provided at home also under proper supervision and vital monitoring.

Results

This study is significant in treatment of COVID-19 patients. Looking to severity of disease, it is important to develop right approach through which prone patients to severe symptoms and complications can be identified and given extra care. The findings of this study tell that CBC and biochemical parameters play important role in determination of COVID-19 patients ICU requirements or not. According to this study the laboratory parameters that need to be determining for ICU care are S.Ferritin and NLR. Out of 25 ICU patients, 100% patients had increased NLR, 84% had increased d-dimer, 79% had increased S.ferritin, 80% had raised CRP and 71% had increased S.Creatinine. Out of 76 Non-ICU patients only 18.42% had raised NLR, 19% had raised S.ferritin, 23.2% had raised d-dimer, 30% had raised CRP and 11.45% had raised S.Creatinine. However A: G ratio and LDH values did not vary significantly between ICU and Non ICU Covid positive patients. This has been decided on the basis of ROC Curve, which were observed to be very good test. The next most preferred tests are CRP and D-dimer ROC curve, which were observed as fair tests. The tests of LDH and A: G ratio should be avoided as they are poor tests.

Limitations

It should be noted that there are some limitations are there in interpretation of findings of the study.

1. Samples used in study have unequal number of males and females.
2. It should also be taken in consideration that this study did not control for effect of pre existing health conditions.
3. It is important for future studies to determine whether predicted observations occurred independently of preexisting health conditions like DM, Hypertension etc. or not.

Conclusion

Above all limitations, this study has provided insights into laboratory parameters which can be used to predict the severity of COVID-19 cases. We concluded that the area under ROC curve of the assessed biochemical parameters and CBC parameters. The biochemistry with very good accuracy was NLR and S.Ferritin, with good accuracy were D-dimer, S.Creatinine and CRP.

It is evident the severity of COVID-19 patients and should receive ICU care if raised. It is therefore recommended that healthcare providers should initiate the use of highlighted parameters in making decision regarding patient management especially when there are limits ICU facilities.

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