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Original Research Article

The Role of Biomarkers in Diagnosis and Risk Stratification for COVID-19 Deterioration, Death

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Abstract:
A Retrospective observational study was done to identify role of laboratory biomarkers in diagnosis and risk stratification for COVID-19 deterioration, death in a tertiary care hospital. A total of 70 COVID-19 patients were included in the study after having applied inclusion and exclusion criteria. Two groups were made survival (40) and non-survival (30). Maximum number of non–survival were in the age group of 66-75 years (33.33%). This study investigate role of Laboratory biomarkers- LDH, Ferritin, D Dimer, PCT, IL6, Urea, Creatinine, SGOT, SGPT that were significantly raised in non-survival COVID-19 patients. Assessing and monitoring the laboratory biomarkers of LDH, FERRITIN, DDIMER, PCT, IL6, UREA, CREATININE, SGOT, SGPT at the earliest stage of the disease could have considerable role in halting disease progression and death.

Keywords: COVID-19, Biomarkers, LDH, Feritin, Ddimer, PCT, IL6, Urea, Creatinine, SGOT, SGPT.

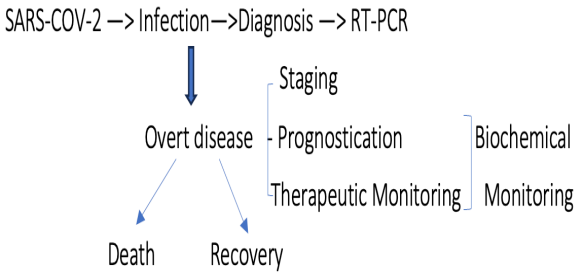
Introduction

A Retrospective observational study was done to identify role of laboratory biomarkers in diagnosis and risk stratification for COVID-19 deterioration, death in a tertiary care hospital. A total of 70 COVID-19 patients were included in the study after having applied inclusion and exclusion criteria. Two groups were made survival (40) and non-survival (30). Maximum number of non–survival were in the age group of 66-75 years (33.33%). This study investigate role of Laboratory biomarkers- LDH, FERRITIN, DDIMER, PCT, IL6, UREA, CREATININE, SGOT, SGPT that were significantly raised in non-survival COVID 19 patients. Assessing and monitoring the laboratory biomarkers of LDH, FERRITIN, DDIMER, PCT, IL6, UREA, CREATININE, SGOT, SGPT at the earliest stage of the disease could have considerable role in halting disease progression and death.

In severe cases, there is an over -reaction of immune system (cytokine storm) which leads to eventual death. Such patients have higher plasma concentration of inflammatory cytokines such as IL-6. The viral spike protein acts like hepcidin causing ferroportin blockage leading to iron overload. Desaturation of hemoglobin leads to iron overload in tissues as hyperferritinemia, the release of free circulating heme, systemic hypoxia, reduction in nitric oxide, and finally activation of coagulation causing thromboembolic episodes.

Biochemical test for monitoring

The critical role of laboratory medicine in COVID -19



Laboratory biomarkers are critical for assessing disease severity and progression as well as monitoring therapeutic intervention. Inflammation can lead to multi-organ failure. Liver dysfunction is common in COVID 19 patients. Acute kidney injury is risk factor for mortality.

Inflammatory markers

Cytokine storm is suspected when the patients has shortness of breath/high fever/ hyperferritinemia/ high CRP. The pro- inflammatory cytokines such as IL6 are increased.

Biochemical markers of inflammation, including ferritin, PCT, CRP have been elevated in COVID-19.

D-Dimer is the degradation product of fibrin clot and marker for blood clotting. The elevation of D-Dimer is associated with worsening of disease, increase chances of thromboembolism, deep vein thrombosis, pulmonary embolism, and disseminated intravascular coagulation.

D- Dimer above 1 microgram/l and CRP above 125 milligram/l indicate poor prognosis

Ferritin level 500-1000 nano gram/ml is indicative of hyper inflammation.

Aims and Objectives of the Study

To identify laboratory biomarkers that predicts disease severity among COVID-19 patients.

Material and methods

A retrospective observational study was done in the department of biochemistry, GMC Amritsar. Prior approval from ethical committee was taken. A total of 70 COVID-19 patients, aged 20-85 years admitted to Government Medical College Amritsar from June 2020
to June 2021 were enrolled in the study.

Inclusion Criteria

All the RT-PCR Positive patients admitted to COVID wards of Guru Nanak Dev Hospital were included in the study. Laboratory investigations and other detailed records of these patients was recorded from the files. The outcomes of the disease i.e. stay in hospital, condition on discharge or death was recorded.

The laboratory investigation which were taken into consideration include:

1. Liver function test
2. Kidney function test
3. D-Dimer
4. Procalcitonin
5. IL-6
6. Ferritin
7. LDH
8. CRP

Statistical Analysis

Data is described in terms of range; mean ± standard deviation (μ ± SD), median, frequencies (number of cases) and relative frequencies (percentages) as appropriate. To determine whether the data is normally distributed, a Kolmogorov-Smirnov test was used. Comparison of quantitative variables between the study groups was done using Mann Whitney U test for independent samples for non-parametric data. For comparing categorical data, Chi square ($\chi 2$) test was performed and fisher exact test was used when the expected frequency was less than 5. A probability value (p value) < 0.05 was considered statistically significant. Every statistical calculation was done using (Statistical Package for the Social Science) SPSS 21version (SPSS Inc., Chicago, IL, USA) statistical program for Microsoft Windows.

Results and Observation

In this study, the study population was divided into two groups, survival and non-survival. Out of 70 patients, 30 were non survival.

Among the non-survival, 33.33% of patients were in the age group of 66-75 years, 20% of patients were in the age group of 56-65 years, 13.33% of patients were in the age group 46-55 years and 76-85 years, 10% of patients each were in the age group 25-35 years and 36-45 years. So maximum number of patients were in the age range of 66-75 years among non-survival.

Table 1: Age distribution among non-survival

Age-group	No of patients	Percentage
25-35	3	10
36-45	3	10
46-55	4	13.33
56-65	6	20
66-75	10	33.33
76-85	4	13.33

Various bio markers that were statistically significant among non-survival were LDH, Ferritin, D Dimer, PCT, IL6, Urea, Creatinine, Sgot, SGPT.

The mean LDH level was 1154.63 ± 726.65 IU/L in survival patients and in non-survival mean LDH level was 1542.07 ± 631.69 IU/L. Results were statistically significant (p- 0.021)

The mean ferritin level was 524.37 ± 344.85ng/ml in survival patients and in non-survival mean ferritin level was 1038.40 ± 833.0ng/ml. Results were statistically significant (p- 0.003)

The mean D Dimer levels was 5.73 ± 5.99μg/ml in survival patients and in non-survival mean D Dimer level was 14.68 ± 22.65μg/ml. Results were statistically significant (p- 0.016)

The mean PCT level was 5.70 ± 18.63ng/ml in survival patients and in non-survival mean PCT level was 11.15 ± 36.56ng/ml. Results were statistically significant (p- 0.027)

The mean IL6 level was 98.14 ± 110.22pg/ml in survival patients and in non-survival mean IL6 level was 253.50 ± 496.38pg/ml. Results were statistically significant (p- 0.045)

The mean urea level was 63.95 ± 62.14mg/dl in survival patients and in non-survival mean urea level was 112.57 ± 104.91mg/dl. Results were statistically significant (p- 0.001)

The mean creatinine level was 1.68 ± 1.38mg/dl in survival patients and in non-survival mean creatinine level was 3.41 ± 4.26mg/dl. Results were statistically significant (p- 0.002)

The mean SGOT level was 42.65 ± 34.33IU/L in survival patients and in non-survival mean SGOT level was 86.13 ± 125.24IU/L. Results were statistically significant (p- 0.004)

The mean SGPT level was 35.93 ± 32.07IU/L in survival patients and in non-survival mean SGPT level was 69.73 ± 94.53IU/L. Results were statistically significant (p- 0.007)

The mean CRP level was 29.86 ± 34.49mg/dl in survival patients and in non-survival mean CRP level was 34.49 ± 46.29mg/dl. Results were statistically insignificant (p- 0.629). ALP (p-0.057) and Total bilirubin (p-0.622) levels were statistically insignificant.

Table 2: Biomarkers in COVID-19 Patients

	Survival		Non survival		Z	p-value
	Mean	SD	Mean	SD		
LDH	1154.63	726.65	1542.07	631.69	-2.362	0.021
CRP	29.86	35.36	34.49	46.29	-0.485	0.629
Ferritin	524.37	344.85	1038.40	833.07	-2.938	0.003
D Dimer	5.73	5.99	14.68	22.65	-2.479	0.016
PCT	5.70	18.63	11.15	36.56	-2.209	0.027
IL6	98.14	110.22	253.50	496.38	-1.989	0.045
S Urea	63.95	62.14	112.57	104.91	-3.662	0.001
S Creatinine	1.68	1.38	3.41	4.26	-3.077	0.002
OT	42.65	34.33	86.13	125.24	-2.883	0.004
PT	35.93	32.07	69.73	94.53	-2.721	0.007
ALP	93.79	25.24	140.33	100.83	-1.901	0.057
BIL	0.74	0.46	1.18	2.53	-0.494	0.622

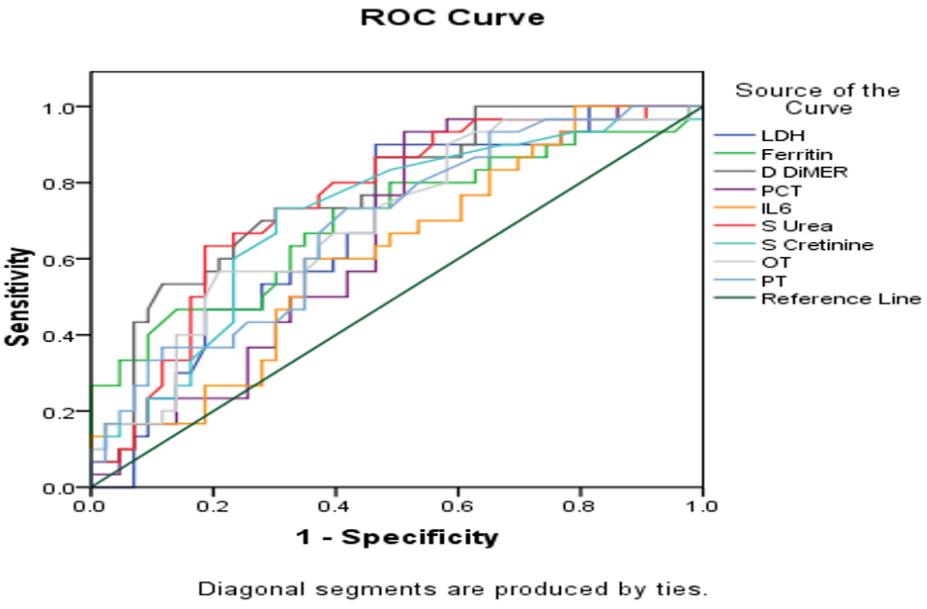


Table 3: Area Under The Curve

Test Result Variable(s)	Area	Std. Error[a]	p-value	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
LDH	0.683	0.063	0.008	0.560	0.806
Ferritin	0.703	0.064	0.003	0.578	0.828
D Dimer	0.773	0.055	0.000	0.666	0.880
PCT	0.653	0.064	0.027	0.527	0.778
IL6	0.617	0.066	0.090	0.488	0.746
S Urea	0.753	0.057	0.000	0.641	0.866
S Creatinine	0.712	0.062	0.002	0.591	0.834
OT	0.699	0.062	0.004	0.578	0.820
PT	0.688	0.062	0.007	0.567	0.809

As depicted by ROC curve and Table 3, the parameters are specific and show sensitivity for outcome of the patient suffering from COVID-19.

Discussion

COVID 19 first case was identified in Wuhan, China and it is associated with high morbidity and mortality.

In the present study the mean LDH levels was 1154.63 ± 726.65IU/L in survival patients and in non-survival mean LDH levels was 1542.07 ± 631.69IU/L. These values were statistically significant (p- 0.021)

3 studies had compared elevated LDH values with survival and non-survival in 514 patients, 157 (30.5%) of whom were non-survivors [1]. 151 patients who had elevated LDH values were non-survivors (96.2%) out of 354 patients (68.9%). 203 patients (56.9%) were in the survivor group. The LDH range in the included studies were 245 to 253.2 U/L.

The mean ferritin levels was 524.37 ± 344.85ng/ml in survival patients and in non -survival mean ferritin levels was 1038.40 ± 833.07ng/ml. These values were statistically significant (p- 0.003).

Hyperferritinemia is caused by the excessive inflammation due to the infection. It is associated with the admission to the intensive care unit and had high mortality. High ferritin level represents an indication to recognize high-risk patients to guide the therapeutic intervention to control inflammation. Serum ferritin, a feature of hemophagocytic lymphohistiocytosis, which is a known complication of viral infection, it is closely related to very poor recovery of COVID-19 patients. Those with impaired lung lesion are more likely to have increased ferritin levels. [2] As a pro-inflammatory factor in the uncontrolled cytokine storm, ferritin level is predictive of poor outcome in COVID-19 patients

The study by Yan et al revealed higher ferritin levels in severe patients.[2] Lu et al reported that COVID-19 patients with high levels of ferritin have greater proportions of severe and deceased cases (P = .0016).[3]

Zhou et al concede that the increase in ferritin level is associated with the worsening of the COVID-19. [4] The cytokine storm and the fabricated host immune response (i.e, ferritin) participate in the development of ARDS, which is the leading cause of mortality if it progresses to respiratory failure.

The mean D Dimer levels was 5.73 ± 5.99µg/ml in survival patients and in non-survival mean D Dimer levels was 14.68 ± 22.65µg/ml. Results were statistically significant (p- 0.016)

Guan et al. revealed that severe patients had a significantly higher level of D-dimer than non-severe patients by analysing 1,099 patients with laboratory-confirmed COVID-19 from all over 550 hospitals in China [5]. Moreover, Zhou and his colleague had done a retrospective study involving 191 COVID-19 patients and found that elevated D-dimer at admission was a risk factor for death of adult patients[6]

The mean PCT levels was 5.70 ± 18.63ng/ml in survival patients and in non- survival mean PCT levels was 11.15 ± 36.56ng/ml. These values were statistically significant (p- 0.027)

Several studies have reported that elevated PCT levels are positively associated with the severity of COVID-19. A meta-analysis also demonstrated that increased PCT values are linked to an ~5-fold higher risk of severe SARS-CoV-2 infection [7-11]

The mean IL6 levels was 98.14 ± 110.22pg/ml in survival patients and in non- survival mean IL6 levels was 253.50 ± 496.38pg/ml. These values were statistically significant (p- 0.045)

Several studies proclaimed that elevated IL6 levels are significantly associated with severity of COVID-19. Analysis revealed 2.9-fold higher levels in

patients with complicated Covid-19 as compared with non -complicated COVID 19 disease (six studies; n = 1302; 95%CI, 1.17-7.19; I2 = 100%).[12]

The mean urea levels was 63.95 ± 62.14mg/dl in survival patients and in non- survival mean urea levels was 112.57 ± 104.91mg/dl. These values were statistically significant (p- 0.001)

The mean creatinine levels was 1.68 ± 1.38mg/dl in survival patients and in non- survival mean creatinine levels was 3.41 ± 4.26mg/dl. These values were statistically significant (p- 0.002)

Urea and Serum creatinine (the relevant indicator of AKI) levels were higher in severe COVID-19 patients than in patients of other commonly known pneumonia.[13] The occurrence of kidney dysfunctions in COVID-19 patients might be explained by the kidney-lung crosstalk theory [14-17] because of the following reasons. First, the SARS-CoV-2 uses ACE2 (angiotensin-converting enzyme 2) as a cell entry receptor which was not exclusively expressed in the respiratory organs, kidney and with a much higher level than that in the lung. SARS-CoV-2 could also attack renal tubular epithelial cells in addition to attacking lung epithelial cells [18]. Pre-printed report analysed the histology of renal tissues from autopsies and found acute renal tubular damage in six COVID-19 cases [19]

The mean SGOT levels was 42.65 ± 34.33IU/L in survival patients and in non- survival mean SGOT levels was 86.13 ± 125.24IU/L. Results were statistically significant (p- 0.004)

The mean SGPT levels was 35.93 ± 32.07IU/L in survival patients and in non- survival mean SGPT levels was 69.73 ± 94.53IU/L. Results were statistically significant (p- 0.007)

SARS-CoV-2 Virus could possibly have effect on liver; the virus could directly affect the hepatocytes or the liver could get injured indirectly by increased inflammatory response due to the raised immune markers and drug toxicity that are meant to treat or stop the progression of the disease resulting in liver damage and thereby raised liver enzymes [20]. Raised liver enzymes is associated with more severe disease category and is also reported in other studies [21-26].

The mean CRP levels was 29.86 ± 34.49mg/dl in survival patients and in non- survival mean CRP levels was 34.49 ± 46.29mg/dl. These values were statistically insignificant (p- 0.629). ALP (p-0.057) and Total bilirubin (p-0.622) levels were statistically insignificant.

Our study had some limitations. This study was conducted at a single-center with limited sample size.

Conclusion

This study highlights that laboratory biomarkers such as LDH, Ferritin, D Dimer, PCT, IL6, Urea, Creatitine, SGOT, SGPT are significantly raised in non-survival as compared to survival.

This study showed that LDH could be identified as early laboratory marker of lung injury and severe COVID-19 cases.

Raised Ferritin levels was associated with bad prognosis and could predict the worsening of COVID-19 patients.

D-dimer levels is linked to poor prognosis. Early measurement of D-dimer values can also be useful in controlling and managing COVID-19 disease.

This study demonstrates that PCT is an indicator of disease severity among COVID-19 patients. Serial PCT measurements may be useful in predicting the prognosis.

Raised Levels of IL-6 in severe COVID 19 patients had adverse clinical outcomes. Inhibition of IL-6 may be a novel target for therapeutics for the management of rampant host responses in patients with Covid-19

Although lung is primarily affected by SARS-CoV-2 infection but we found in our study that COVID 19 patients exhibit kidney dysfunction.

Raised renal function test in COVID-19 patients is an important negative prognostic factor for survival.

SGOT and SGPT levels were significantly raised in non- survival patients. Raised SGOT and SGPT levels were found to be significant predictors of developing severe COVID-19 disease.

In our study CRP, ALP, TOTAL BILIRUBIN levels were insignificant. In some patients Levels may be raised but that are insignificant.

The study can serve as database for future comparisons and can be useful for clinicians. Assessing and monitoring the laboratory biomarkers at the earliest stage of disease could have considerable role in halting disease progression and death.

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