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Utility of biochemical markers in predicting severe COVID-19: experience from a tertiary hospital in South India

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ABSTRACT

Background

Coronavirus Disease 2019 (COVID-19) patients can present with a wide array of symptoms. For laboratory investigation of these patients several biochemical tests are routinely requested. Here we wanted to evaluate the utility of procalcitonin (PCT), ferritin, D-dimer, interleukin 6 (IL-6) and total lactate dehydrogenase (LDH) activity in predicting severe COVID-19 infection.

Patients and methods

This study was undertaken at a tertiary care medical hospital in Tamil Nadu, India representing 183 COVID-19 RT-PCR positive patients, who were grouped based on their disease severity as mild (n=21), moderate (n=115) and severe (n=47) cohorts. All routine

clinical chemistry analysis was performed as part of routine baseline assessment. Biomarkers of inflammation and infection were tested via the measurement of IL-6, PCT, ferritin, and D-dimer. Serum IL-6 concentration was estimated by ELISA, while total LDH activity was analyzed by kinetic colorimetric assay. Serum ferritin, PCT and D-dimer were measured by fluorescent immunoassay by sandwich immuno-detection method.

Results

Biomarkers were significantly different among subgroups, and the highest concentrations were found in those with intensive care unit (ICU) admission. Serum PCT showed the best power to predict the need for ICU treatment followed by D-dimer, IL-6 and total LDH. Based on the AUC-ROC analysis, mortality was most effectively indicated by D-dimer followed by PCT, LDH, IL-6 and ferritin.

Conclusion

Our study highlights the utility of some routinely available biochemical tests in the management of severe COVID-19. The higher baseline values of these biomarkers hint towards the probability of severe infection and a larger risk of death.

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INTRODUCTION

Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2) has gripped the world after being first reported in Wuhan, China in December 2019. An enveloped single stranded RNA virus belonging to the family Coronaviridae and subfamily of orthocoronavirinae was isolated as the cause of the pandemic [1,2]. Since millions of people across the globe have been prey to this infection and have succumbed due to it. A highly populous country like India with low sanitation levels has been an easy target. The Coronavirus Disease 2019 (COVID-19) patients can present with a wide array of symptoms, which include mild fever, cough, fatigue, upper respiratory symptoms and gastrointestinal symptoms. Anosmia and dysgeusia have been reported to be frequently found in these patients. Some cases can develop severe complications, such as Acute Respiratory Distress Syndrome (ARDS), respiratory and cardiac failure leading to multiorgan dysfunction and death [3]. Early therapeutic intervention and continuous monitoring during therapy play a critical role in reducing mortality.

Evidence accumulated in recent past has suggested the critical role of cytokines and chemokines released due to cellular destruction caused by rapid viral proliferation [4]. The molecular testing forms the basis for diagnosis, but the requirement of sophisticated instruments and unavailability of trained personnel for performing Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) has been challenging. Several biomarkers are being utilized to predict severity of the disease. Inflammatory markers like Procalcitonin (PCT), C-reactive Protein (CRP) and interleukin-6 (IL-6) are being reported to be associated with the severity of COVID-19 infection [5]. Liver enzymes and renal functions are also monitored in patients suffering from COVID-19 [6,7].

Several biochemical tests are being performed in COVID-19 subjects. Risk stratification of COVID-19 cases can be done using the array of biochemical tests available. Hence, it is desirable to find early and effective predictors of clinical outcomes in these patients. Patients with severe COVID-19 presented with an immunochemical profile like in cytokine storm. The intensified production of pro-inflammatory cytokines may be involved in pathophysiology causing severe pulmonary oedema, respiratory failure and damage to organs, such as liver heart

and kidney [8]. Increase in pro-inflammatory cytokines e.g., IL-6 and tumour necrosis factor-α (TNF-α), have been observed in patients with severe disease and found to be significantly associated with mortality [9]. PCT is a routinely used inflammatory marker in the daily routine. Any microbial infection can cause a significant raise in PCT, as endotoxins and pro-inflammatory cytokines induce its release from parenchymal tissues. Various studies have supported the theory that considerable increase in PCT levels from its baseline value denotes the beginning of critical phase of COVID-19 infection [10]. Formation and lysis of cross-linked fibrin gives rise to D-dimer. This reflects the activation of coagulation and fibrinolysis. Severity of COVID-19 symptoms are found to be associated with hemostatic abnormalities and elevated levels of plasma D-dimer values [11]. Ferritin, being an acute phase reactant, is linked to the underlying systemic vasculitis that cause lesions in major organ systems [12]. Lactate dehydrogenase enzyme (LDH) is present in numerous tissues throughout the body; thus, tissue damage easily leads to its serum elevation. LDH in COVID-19 cases is seen as a marker of lung injury in the initial stage of the disease [13].

The plethora of pathological processes in COVID-19 include hyperinflammation, cytokine storm, dysregulation of coagulation pathway, thereby producing a picture of systemic vasculitis leading to varied fatal complications. Our study was to assess the utility of widely used biochemical parameters in predicting the severity and mortality in COVID-19 infection. We aimed to define the relative cut-off values for various biomarkers to foretell disease morbidity in COVID-19 infected individuals.

MATERIALS AND METHODS

This clinical study was carried out by the Department of Biochemistry, in a tertiary care medical college hospital located in Madurai, India. Consecutive adult patients with positive RT-PCR results were enrolled at this hospital from August 2020 to October 2020. The study was approved by the Institutional ethics committee. This study is in compliance with the ethical principles for medical research involving human subjects, in accordance with the Declaration of Helsinki. The patients were grouped according to their clinical symptoms into mild cases as group I, moderate cases were grouped as group II and severe cases were grouped as group III, based on National Clinical Management Protocol COVID-19, Revised version 3, dated June 13, 2020, by the Ministry of Health and Family Welfare, Government of India. According to the guideline, patients with uncomplicated upper respiratory tract infection, and mild symptoms, such as fever, cough, sore throat, nasal congestion, malaise and headache were categorized as mild cases, who could be managed at home. Pneumonia with no signs of severe disease with presence of clinical features of dyspnoea and or hypoxia, fever, cough, including SpO_2 <94% (range 90-94%) on room air, respiratory rate more or equal to 24 per minute were categorized as moderate cases. Severe cases were those patients who developed severe pneumonia or ARDS with severe hypoxia. Patients who presented with sepsis or acute life-threatening organ dysfunction caused by an unregulated host response to suspected or proven infection were considered as severe cases. Patients presenting with persisting hypotension despite volume correction or even after correction with vasopressors were also grouped as severe cases [14].

Exclusion criteria

Subjects showing negative RT-PCR results for COVID-19, or having a history of any hepatic and renal diseases prior to being infected with viral pneumonia were excluded. Pregnancy and

the presence of malignancy were exclusion criteria as well.

Inclusion criteria

All adults, who were tested for COVID-19 infection and had positive result by RT-PCR during the defined study period were included into the study.

Assignment of study group

Patients in the mild group I were treated with home quarantine. The moderate cases (group II) were admitted to the hospital and were treated in isolation wards. The severe cases assigned to group III required admission to intensive care unit (ICU). The patients were sub-grouped as survivors and non-survivors based on the mortality at the time of discharge from the health care facility for further analysis.

SARS-CoV-2 RT-PCR testing was done by a closed system, Truenat from Molbio diagnostics private limited, India on Truelab workstation. Qualitative detection of SARS-CoV-2 was done from upper respiratory specimens (nasopharyngeal swabs and oropharyngeal swabs) in our hospital. Results were calculated based on graphical analysis and cycle threshold (Ct) values. The Envelope (E) gene and Open Reading Frame-1 (ORF1) gene were targeted for detection of infection by commercially available kit, as per manufacturer's instruction [15].

Data collection

Clinical data included gender, age, time of admission and time of discharge. Routine biochemical and hematological tests were conducted to assess their baseline values. All routine clinical chemistry analysis like renal and liver functions, serum electrolytes, complete blood count were performed as part of the routine baseline assessment. The routine clinical chemistry tests were performed using Toshiba 120FR fully automated system for baseline assessment of the patients. Biomarkers of inflammation and infection were tested, which consisted of IL-6, PCT, ferritin, and D-dimer. Serum IL-6 was estimated using a commercially available human IL-6 ELISA kit (Biotech Diaclone, Besançon, France) with a sensitivity of 2 pg/mL as per the manufacturer's instructions [16]. Serum ferritin, PCT and D-dimer were measured by fluorescent immunoassay by sandwich immuno-detection method using i-Chroma analyzer [17]. Total LDH activity was analyzed by kinetic colorimetric assay.

Statistical analysis

Data was analyzed using IBM SPSS v.16.0 statistical software. The non-normal distribution was confirmed by subjecting data for Kolmogorov-Smirnov test. Continuous variables with non-parametric distribution were expressed as the median (25th percentile, 75th percentile). Mean values with standard deviation were used to express data that was continuous and equally distributed. The categorical variables were summarized as frequencies and percentages. The data were compared between the groups based on severity of COVID-19 infection by using ANOVA and K independent sample test for parametric and non-parametric distribution, respectively.

Students unpaired t-test and Mann-Whitney U test were used for two-group comparisons of continuous variables in different groups based on the mortality. Statistical significance was assumed if $p < 0.05$. Receiver Operating Characteristic (ROC) curve analysis was performed to determine the diagnostic utility of various biomarkers of COVID-19 for determining ICU admission and for predicting mortality. The measures of diagnostic accuracy including the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio and negative likelihood ratio were calculated using MedCalc's diagnostic test evaluation calculator [18].

RESULTS

A total of 183 patients were included into the analysis, 69% (n=126) were males and 31% (n=57) were females. The patients were divided based on the severity of their disease as group I (n=21), group II (n=115) and group III (n=47). The mean age of the study population was 57.89 ± 14.3 years. Amongst our study population, 19.6% (n=36) died of the disease. There was no casualty in group I. Group II with moderate cases had a mortality rate of 10%, i.e., eleven cases. 53% of all cases (25 cases) from group III constituting severe cases, died. All statistical analyses and conclusions drawn are based on baseline values of the parameters studied. Table 1 shows the distribution of age, gender and biochemical markers amongst the three groups. All parameters showed difference across the 3 groups. P values for baseline characteristics and biochemical markers between the three groups are depicted in Table 1. All biomarkers were distributed in a statistically significant ($p<0.05$) manner amongst the groups. There was a statistical

Table 1 Distribution of baseline characteristics and biochemical markers of COVID-19 infected patients based on the severity of the infection

	Biological reference interval	Group I n=21	Group II n=115	Group III n=47	p value
Age (years)		47 ± 15	57 ± 14	62 ± 12	**<0.001***
Males [N (%)]		10 (48%)	88 (77%)	28 (60%)	
Total Protein (g/dl)	6-7.8	6.8 ± 0.6	6.4 ± 0.7	6.1± 0.8	**0.003***
Albumin (g/dl)	3.5-5.5	4 ± 0.3	3.7 ±0.4	3.6 ± 0.6	**0.002***
Sodium (mEq/L)	136-145	137 ± 4	135 ± 4	133 ± 6	**0.009***
Potassium (mEq/L)	3.5-5.0	3.9 ± 0.4	4.1 ± 0.6	4.3 ± 0.8	0.203
Chloride (mEq/L)	98-106	84 ± 42	100 ± 10	100 ± 6	0.857
Aspartate Transaminase (U/L)	Less than 35	30 (24, 58)	40 (34, 60)	51(35,69)	0.175
Alanine Transaminase (U/L)	Less than 35	24 (21, 36)	33(23, 53)	33 (25,56)	0.717
Alkaline Phosphatase (U/L)	36-92	80(69, 95)	73(59,101)	83 (64,106)	0.357
Urea (mg/dl)	17-43	22 (17, 27)	30(23, 42)	44 (29, 68)	**<0.001***
Creatinine (mg/dl)	0.7-1.3	0.8 (0.6, 0.85)	0.8 (0.6, 1)	1.0 (0.8,1.4)	**0.007***

Procalcitonin (ng/ml)	<0.1	0.1 (0.1, 0.1)	0.1 (0.1, 0.2)	0.3 (0.1, 0.6)	**< 0.001***
D-dimer (ng/ml)	<500	212 (165, 251)	382 (203, 743)	818 (368, 4490)	**< 0.001***
Interleukin 6 (pg/ml)	5.3 - 7.5	6.9 (5.1, 10.6)	50.8 (11.4,172.7)	144 (63.5, 32605)	**< 0.001***
Ferritin (ng/ml)	M: 20-250 F: 10-120	43 (18, 130)	328 (136, 536)	442 (188, 686)	**< 0.001***
Total lactate dehydrogenase (U/L)	60-100	514 ± 170	837 ± 378	1055 ± 539	**0.001***

*Notes: Data are mean ± SD and median (25th Percentile, 75th Percentile). *p <0.05 is significant, M: Males, F: Females.*

significance in the age distribution across the 3 groups, with older individuals having a higher disease severity.

The AUC-ROC curves were used for comparing the potential of different biomarkers such as PCT, D-dimer, IL-6, Ferritin and LDH to predict severity and mortality due to COVID-19, respectively (Figures 1 and 2). Accordingly, serum PCT had the best power to predict ICU admission followed by D-dimer, IL-6 and LDH.

Figure 1 **Receiver operator characteristic curves comparing the potential of biochemical markers to predict severity of COVID-19**

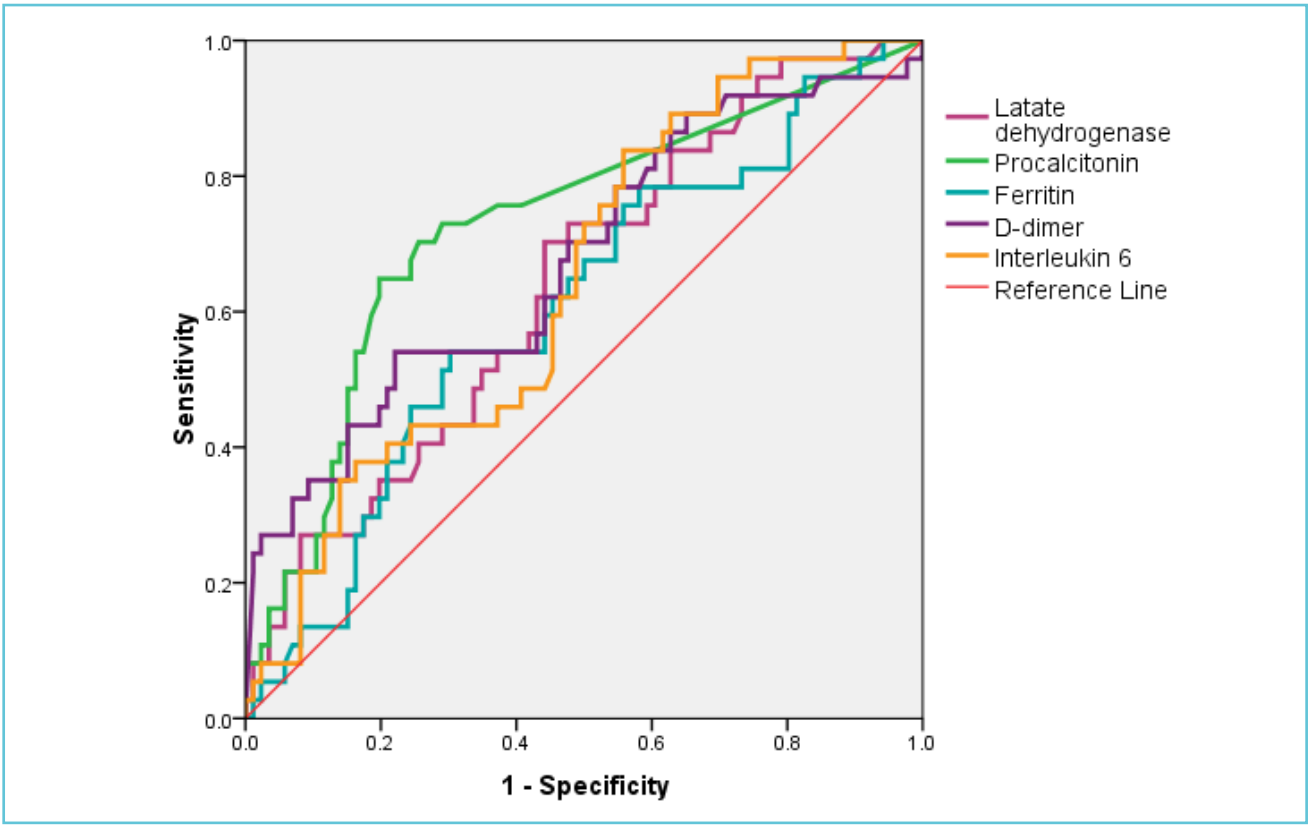
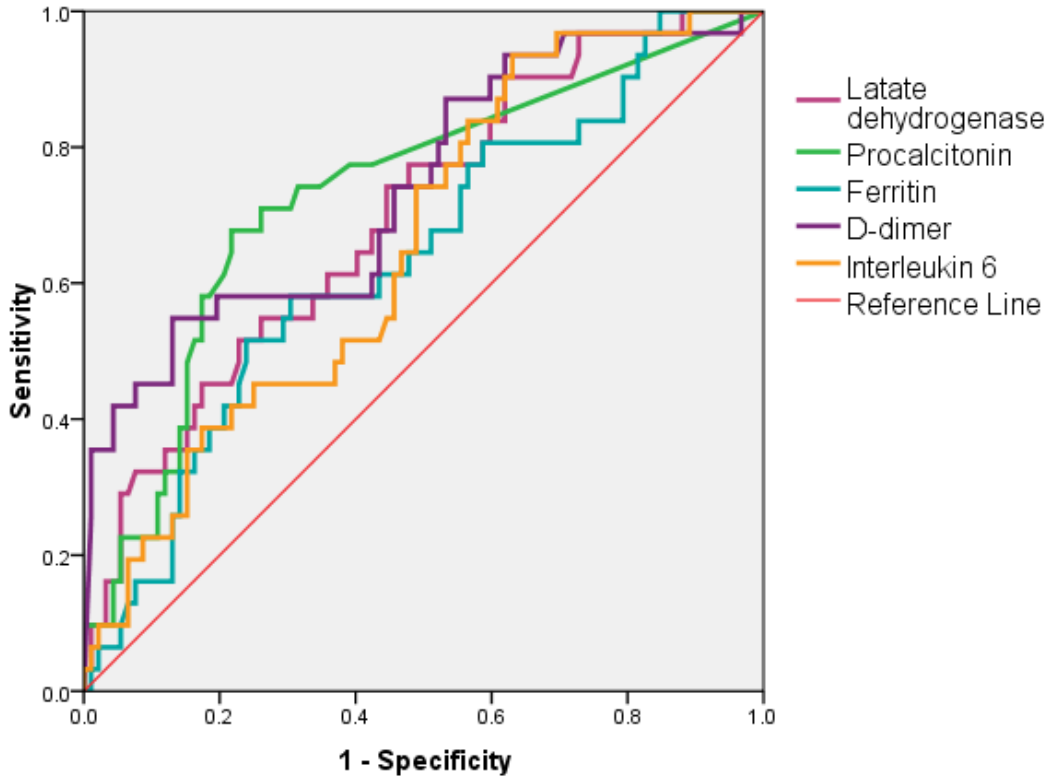


Figure 2 Receiver operator characteristic curves comparing the potential of biochemical markers to predict the mortality in cases infected with COVID-19



Based on ROC curves of biomarkers, comparison to predict mortality was done by analyzing the measures of diagnostic accuracy as displayed in Table 2. The ROC curve was used to obtain a specific cut-off for each biomarker. PCT had a sensitivity of 71% and a specificity of 70.7% at 0.15 ng/ml. Ferritin had a sensitivity of 58.1% and a specificity of 56.5% at 448 ng/ml. IL-6 showed a sensitivity of 74.2% and a specificity of 44.6% at 60 ng/ml, while D-dimer had a sensitivity of 58.1% and a specificity of 70.7% at 684 ng/ml. Finally, total LDH had a sensitivity of 77.4% and a specificity of 52.2% at 794 U/L (Figure 1).

PCT and D-dimer are seen to have better performance in comparison to IL-6, LDH and ferritin with respect to their AUC-ROC (Figure 1 and Table 2). D-dimer is seen to have best NPV followed by PCT to predict mortality. IL-6 was seen to have the highest PPV to predict mortality. The positive likelihood ratio for mortality prediction was seen to be best with IL-6 followed by PCT.

The distribution of biochemical markers among COVID-19 patients grouped based on their outcome are presented in Table 3. We found non-survivors to be significantly older than survivors.

DISCUSSION

This study is a retrospective study which was conducted to analyze the usefulness of some routinely available biochemical markers in the management of COVID-19 infection. Patients infected with SARS-CoV-2 infection tend to develop ARDS which requires early detection and monitoring from initial stages to prevent poor outcomes.

Table 2 Diagnostic performance of different biomarkers based on their cut-off value from the ROC curve analysis for the prediction of mortality in COVID-19

Biomarker (Cut-off)	AUC (95% CI)	p value	PPV (95% CI)	NPV (95% CI)	Positive Likelihood Ratio (95% CI)	Negative Likelihood Ratio (95% CI)
PCT (0.15 ng/ml)	0.731 (0.625, 0.838)	**<0.001***	71.43% (56.39-82.86)	70.25% (65.45-74.64)	3.89 (2.01-7.53)	0.66 (0.53-0.82)
Ferritin (448 ng/ml)	0.637 (0.525, 0.749)	**0.022***	55.56% (41.36 - 68.9)	62.61% (57.94-67.05)	1.75 (0.99 –3.09)	0.83 (0.69 -1.01)
IL-6 (60 pg/ml)	0.656 (0.551, 0.760)	**0.010***	80.56% (65.67-89.97)	58.22% (54.43-61.91)	4.23 (1.96-9.17)	0.73 (0.63-0.86)
LDH (794 U/L)	0.699 (0.594, 0.803)	**0.001***	72.22% (57.54-83.3)	56.07% (51.21-60.82)	2.49 (1.3-4.78)	0.75 (0.62-0.91)
D-dimer (684 ng/ml)	0.741 (0.636, 0.847)	**<0.001***	58.33% (44.7-71.24)	72.57% (67.55-77.07)	2.61 (1.48-4.62)	0.71 (0.56-0.90)

Abbreviations: AUC, area under the curve; ROC, receiver operator characteristic; positive predictive value (PPV), negative predictive value (NPV), PCT, Procalcitonin; LDH, Lactate dehydrogenase; IL-6, Interleukin 6.
**p <0.05 is significant.*

Table 3 Distribution of biochemical markers of COVID-19 infected patients grouped based on the outcome

	Survivors (N=147)	Non-Survivors (N=36)	t/z value	p value
Age (years)	56 ± 14	63 ± 10	-2.502	**0.013***
Total Protein (g/dl)	6.4 ± 0.7	6.2 ± 0.2	1.578	0.117
Albumin (g/dl)	3.7 ± 0.4	3.6 ± 0.4	1.337	0.183
Sodium (mEq/L)	135 ± 4	133 ± 7	2.270	**0.025***

Potassium (mEq/L)	4.1 ± 0.5	4.4 ± 0.8	-2.376	**0.019***
Aspartate Transaminase (U/L)	40 (31, 57)	54(40, 78)	-2.357	**0.018***
Alanine Transaminase (U/L)	31 (23, 49)	39(27, 64)	-1.565	0.118
Alkaline Phosphatase (U/L)	74(59, 102)	86(64, 101)	-0.986	0.324
Urea (mg/dl)	29(22, 41)	47(30, 71)	-4.552	**< 0.001***
Creatinine (mg/dl)	0.8 (0.6,1)	0.9(0.8, 1.3)	-2.184	**0.029***
Procalcitonin (ng/ml)	0.1(0.1, 0.2)	0.3(0.1, 0.7)	-4.486	**< 0.001***
D-dimer (ng/ml)	327(195,671)	1638(439, 9477)	-4.985	**< 0.001***
Interleukin 6 (pg/ml)	31.1(9.79, 164.4)	145(76.6, 312.3)	-4.371	**< 0.001***
Ferritin (ng/ml)	241(94,519)	619(313, 768)	-3.334	**0.001***
Lactate dehydrogenase (U/L)	820 ± 375	1133 ± 576	-3.714	**< 0.001***

*Note: Data are mean ± SD and median (25th Percentile, 75th Percentile). *p <0.05 is significant.*

Our study aimed at finding the utility of biomarkers for detecting severity of disease and predict disease outcome. We compared various biochemical tests among sub-groups based on disease severity. Five candidate biomarkers (Ferritin, PCT, IL-6, LDH and D-dimer) were chosen for comparison of their ability to predict severity and morality due to COVID-19 infection. We found that D-dimer and IL-6 had a vast difference between mild and severe cases. Similar finding was seen between survivors and non-survivors. PCT and D-dimer had a higher AUC-ROC curve for predicting severity and mortality as compared with other biomarkers.

Several studies have established that COVID-19 infected patients presented with pneumonia like symptoms [19,20,21]. The blood studies in COVID-19 infected cases at our tertiary care hospital revealed elevation of various inflammatory markers, such as IL-6, ferritin, D-dimer and PCT, which were comparable to previous reports [5,21,22]. ARDS associated with vast production of inflammatory cytokines, resulting in multi-organ dysfunction in viral infections resembles the features of secondary hemophagocytic lymphohistiocytosis (HLH) [23]. Such proinflammatory response due to exuberant elevation of cytokines has been previously documented in COVID-19 infections [24].

IL-6 is a pleiotropic cytokine, secreted by cells of innate and adaptive immune system as a response to microbial antigens. It causes enhanced activity of T and B cells, neutrophils and monocytes by triggering JAK2-STAT pathway. It induces the secretion of CRP, which helps in activation of classical complement pathway, thereby facilitating mediation of phagocytosis. IL-6 has been proposed to be a good marker of

prognosis in COVID-19 [5,25]. IL-6 contributes to the effective host defense against SARS-CoV-2 infection. However, extensive production of IL-6 can lead to cytokine storm which encompasses severe systemic inflammatory response [26]. IL-6 blockade therapy, using humanized anti-IL-6 receptor antibody, tocilizumab has been found to be beneficial in treating COVID-19 infections [25]. In our study, IL-6 levels were found to be elevated significantly in group III (severe COVID-19 infection). This was similar to the findings in a meta-analysis by Henry et al. [27] and Parsons et al. [28] suggesting the use of IL-6 as a biomarker for prognostic monitoring.

Bacterial infections stimulate amplified production of PCT from extrathyroidal tissue. In viral infections increased interferon-γ inhibits PCT production to remain it in normal limits in non-complicated cases of COVID-19 [28,29]. PCT is more likely to make a distinction between bacterial infection and other inflammatory processes than total leucocytes count or CRP levels [30]. We found PCT to be elevated in severe cases of COVID-19 infection as proposed by previous studies [4,30]. PCT is a crucial biomarker which if elevated at the time of hospitalization may be suggestive of severe COVID-19 infection.

Although lungs are the main target organ for COVID-19, kidneys and liver have been frequently affected due to the hyperimmune response caused by the virus [29]. Angiotensin converting enzyme-2 (ACE-2) receptors are known to ease binding of the virus and help in its entry into the cells [31]. ACE-2 receptors are present abundantly in small intestine, heart muscle, kidney, testis and thyroid [5]. The expression of ACE2 receptors on the renal tubules makes them a target organ for the virus [30]. Renal functions were seen to deteriorate with severe infection. The cholangiocytes have a higher expression of ACE2 receptors, thereby making them a suitable target for SARS-CoV-2 resulting in hepatic dysfunction. The mechanism proposed for transitory elevation in transaminases in COVID-19 infection is secondary liver damage due to hyperinflammatory response to infection. This can also be due to hepatotoxic drugs being used in the management of these patients [22]. Previous studies by Ferrari et al. [32] and Kumar et al. [6] claimed significant levels of elevation of transaminases in severe COVID-19 infections, whereas our findings did not show a statistical significance in the levels of transaminases amongst COVID-19 cases.

Serum ferritin, a marker of iron storage in the body, is seen to increase in cases of inflammation, hepatic disorder and malignancy [4]. It has been increased in patients with severe infection due to COVID-19 as a result of associated secondary HLH and cytokine storm [33]. Controlling of availability of iron to pathogens by ferritin plays a significant role in protecting the body against active infection [31]. Increase in ferritin levels is typically in the range of 500-3000 ng/mL. The increase in ferritin levels leads to activation of endothelial cells in the pulmonary vessels. This can cause imbalance in the normal hemostasis, regulation of fibrinolysis and maintenance of permeability of the vasculature. Such imbalance has a function in the development of COVID-19 vasculopathy resulted by inflammation [34]. The lower respiratory tract injury in COVID-19 patients explains elevated LDH levels. LDH being an indicator of lung injury, increases proportional to the severity of infection [26].

Hyper inflammation leading to elevated D-dimer and fibrinogen levels were seen to cause hypercoagulation and various complications such as Disseminated Intravascular Coagulopathy (DIC) [29]. D-dimer levels were seen to be higher in patients with severe infection as compared with milder infection of COVID-19. Such findings have been described earlier by Ponti et al., who suggested the activation of coagulation and secondary hyperfibrinolysis in mortality due to

COVID-19 infection [30]. D-dimer levels indicated thrombosis and elevated Fibrin Degradation Products (FDP) that occur due to thrombolysis [5]. Administration of anticoagulant therapy with low molecular weight heparin has been reported to be associated with a better prognosis due to decreased venous thromboembolism and DIC.

In our study, IL-6 levels were found to be elevated significantly in in non-survivors. Tjendra et al. studied various biomarkers to predict severity and outcomes in COVID-19, stated that patients with IL-6 >10 pg/ml had a concurrent elevation of various other biomarkers. Such candidates were more likely to develop sepsis and eventually die within 3 days of hospital admission [35].

Non-survivors showed higher values of PCT in our study which was similar to the findings of Gao et al. [20] and a meta-analysis by Malik et al. [22]. Haywood and colleagues studied hospitalization and mortality among COVID-19 patients and found that in-hospital mortality was related to abnormal level of biomarkers, such as lactate, creatinine, procalcitonin and platelet count [36]. Regarding laboratory changes in patients with fatal COVID-19, Henry et al. reported that elevation in the levels of certain biomarkers, such as IL-6, ferritin, PCT, LDH and D-dimer were often seen in cases with fatal COVID-19. PCT can serve as a marker of secondary bacterial infection, which could increase the probabilities of fatal outcome [27].

The non-survivors also exhibited increased serum creatinine and urea concentrations as compared with the survivors (Table 3). Non-survivors had significantly higher AST levels than other liver enzymes. AST with dominated increase was stated to reflect real liver injury [35]. Increased cytokine secretion, ACE2 receptor binding affinity of spike protein of the virus could be predominant cause of multiorgan injury in COVID-19 [37].

Elevation of serum ferritin could be either due to leakage from damaged cells or by active secretion from HepG2 cells and macrophages. Ferritin is seen to possess both immunosuppressive and pro-inflammatory effects [38]. The activation of monocyte-macrophage system causing inflammation is a primary cause of elevated serum ferritin. This supports the theory that diabetics are more prone to developing inflammatory storm which indirectly causes rapid worsening and a poor prognosis in COVID-19 patients [39]. Our patients exhibited high levels of ferritin in the severe and non-survivors of COVID-19 infection which was also observed in previous studies by Keddie et al. [29] and Aloisio et al. [40].

Li et al. evaluated the effect of serum LDH at admission and found it to be an independent risk factor for severity and mortality in COVID 19 cases. Under the influence of acute hypoxia or inflammation, due to lung infection, thrombogenesis and organ injury can occur, thereby making LDH an important marker in COVID-19 cases [41]. LDH is released from numerous tissues during death [29]. Bao et al. suggests LDH as a marker related to the risk of death in COVID-19 cases [42]. In our study, we found highest LDH levels in severe cases and among non-survivors.

Severe inflammation and hypoxia due to pneumonia cause activation of coagulation and fibrinolysis resulting in hypercoagulation state leading to DIC and multi-organ dysfunction. Zhang et al. have studied D-dimer in COVID-19 patients and concluded that D-dimer > 2µg/ml at baseline could predict in hospital mortality [11]. In addition, these patients were at a higher risk of developing pulmonary embolism. Malik et al. opined that elevated D-dimer was related to poor outcomes in COVID-19 patients [13]. Presence of prothrombotic milieu in non-survivors of COVID-19 infection could be the cause of elevated D-dimer levels. Patients with

severe infection and non-survivors exhibited higher levels of D-dimer. Thus, D-dimer is a reliable indicator of severity and can indicate outcome of the infection. This finding is supported by Ye et al. who suggested dynamic monitoring of D-dimer in hospitalized COVID-19 cases to monitor the risk of death [21].

Our observations reflect the efficacy of various biochemical markers. Biomarkers were significantly different amongst all groups and those with ICU admission had the highest concentrations. Serum PCT had the best power to predict ICU admissions followed by D-dimer, IL-6 and LDH (Figure 1). The areas under ROC curve was highest for D-dimer to predict the mortality followed by PCT, LDH, IL-6 and ferritin (Table 2). Finally, D-dimer is a better candidate amongst the chosen biomarkers based on its AUC-ROC curve for predicting mortality.

Our study being retrospective in nature is associated with few limitations. The lack of serial monitoring of various biomarkers is a drawback of our study. This was mainly due to the protocol followed at our institute that comprised of baseline laboratory assessment and continuous clinical monitoring. The inadequate knowledge about SARS-CoV-2 during the initial days were reasons behind such practice. Larger prospective studies with clinical correlation will help us to obtain valuable insights in the disease management and patient outcomes. One other limitation was that the study population included patients with comorbidities such as diabetes, hypertension, overweight, etc., which could also influence the severity and mortality of COVID-19. The sample population being heterogeneous in nature adds weightage to the study. We opine periodic monitoring of biomarkers among COVID-19 patients may aid the early detection of worsening of disease status. This can assist in timely escalation of the treatment protocol, which could be potentially lifesaving.

In conclusion, the higher baseline values of these biomarkers hints towards the probability of severe infection and increased mortality. Baseline biochemical markers help in segregation of high-risk cases and improve the management of patients resulting in an overall improvement. Stratification of cases helps in better management of hospital resources, manpower and aids early identification of requirement of ICU care. Our study highlights the utility of biochemical tests in management of COVID-19. The ease of testing makes them suitable for both triaging as well as monitoring of therapy.

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