Exploring the Interplay of Inflammatory Biomarkers in Predicting Disease Severity and Outcomes in COVID-19 Patients: A Comprehensive Retrospective Analysis.

Author: Sk Erfanul Haque[1], Dr. Sukanta Bhadra[2], Dr. Nishith Kumar Pal[3]

[1] Research Scholar, Department of Microbiology, Techno India University, Kolkata, 700091, India.

[2] Assistant Professor, Department of Microbiology, Techno India University, Kolkata, 700091, India.

[3] Professor & Head, Department of Microbiology, Jagannath Gupta Institute of Medical Sciences And Hospital, Budge Budge, Kolkata, 700137, India.

Abstract

The current cross-sectional, retrospective study examines the modulatory roles of inflammatory biomarkers and their effects on COVID-19 severity and prognosis to inform clinical recommendations. The study revealed, from the data of 455 patients, that different degrees of severity were associated with increased inflammatory biomarkers, such as IL-6 and D-dimer, which were identified as potential mortality biomarkers.

Most importantly, we found that age > 75 years, elevated IL-6 and D-dimer are predictive factors for the risk of mortality. Therefore, the study emphasizes the significance of early assessment of severe cases because higher severity groups show longer-hospital-stay and rather high mortality rates. Future research is needed to confirm the presented findings and to identify other biomarkers that may enhance the risk stratification and patient management.

Keywords: COVID-19, inflammatory biomarkers, disease severity, IL-6, CRP, D-dimer, retrospective analysis, prediction.

Introduction: The global battle against SARS-CoV-2, the causative agent of COVID-19, has prompted extensive research into the clinical manifestations and risk factors associated with the virus. COVID-19 harbours a range of symptoms from mild respiratory that may progress to severe life-threatening features like acute respiratory distress syndrome (ARDS) and multi-organ dysfunction. Such biomarkers as IL-6, CRP, and D-dimer are thought to anticipate disease severity and outcome of the illness. Nevertheless, the nature and the degree of correlation between these biomarkers and disease outcomes is still not fully elucidated, therefore, deserves further research.

Methodology: The data of this retrospective study was collected from 455 COVID-19 patients who were admitted in Jagannath Gupta Institute of Medical Sciences and Hospital, Kolkata, India between 4th August 2020 and 11th January 2022. Patients were categorized according to the severity of the disease into low risk, moderate risk, high risk and very high risk. The investigation evaluated clinical and demographic characteristics along with inflammatory biomarkers such as D-dimer, CRP, and IL-6. Basic statistic tests such as ANOVA, Univariate regression analysis and ROC analysis were also used to infer on the relationship between the inflammatory biomarkers and the severity of the disease or death.

Results: The study revealed significant variations in inflammatory biomarkers across severity categories, with IL-6 (ROC curve of IL-6, AUC: 0.611 [0.538–0.684], p=0.04, cut-off value-20.25, specificity of 82.11% and a sensitivity of 84.21%) and D-dimer levels (ROC curve of D-dimer, AUC of 0.980 [0.777–0.902], <p=0.01, cut-off value-1494, specificity of 84.15% and a sensitivity of 87.96 %,) significantly elevated in non-survivors. Age

≥ 60.50 (ROC curve of age, AUC: 0.637 [0.564–0.711], <p=0.01, cut-off value-60.50, specificity of 66.24% and a sensitivity of 59.70 %.) and D-dimer levels >1494 were identified as predictors of mortality, with high specificity and sensitivity. Conversely, CRP, LDH, Ferritin, and WBC levels showed no significant correlation with COVID mortality. This study also identified longer hospital stays and higher mortality rates in severe and critical risk groups, highlighting the importance of early identification and intervention for severe and critical cases.

Conclusion: These biomarkers of inflammation can help in establishing a prognosis and thus they are worth looking into when studying the severity of COVID-19 in patients. Mortality can appropriately be predicted through IL-6 and D-dimer, which also allows for understanding how the patients are faring clinically. However, there is still need for further investigation so as to confirm these findings and find other possible biomarkers.

Limitations and Future Directions: A potential drawback is the retrospective design as well as the single-centre setting of the study which might influence generalization of the results. The number of participants in this study was relatively small and this means that more research with increased sample size and multicentre work is necessary in the future to confirm the results and also to investigate the other biomarkers. Furthermore, there are a requirement of longitudinal studies to determine the changes in the inflammatory biomarkers and how those changes affect the clinical course of the disease

---

Introduction:

Most of the countries across the globe are struggling with SARS-CoV-2 virus (Huang et al., 2020; (X. Chen et al., 2020). The analysis of the complete viral genome showed that it is very similar to a family of coronaviruses closely related to SARS (Li et al., 2020; Phelan et al., 2020). SARS-CoV-2 positive people present with various signs and symptoms such as fever, dry cough, and fatigue, shortness of breath, and in severe cases, develop ARDS and acute cardiac injury(Graham & Baric, 2020; Zhou et al., 2020). Many severe COVID-19 patients end up in the ICU. Doctors keep a close eye on several important indicators, like neutrophil count, D-dimer levels, blood urea nitrogen, creatinine levels, and lymphocyte counts. They also check for positive viral tests and look for specific patterns on lung CT scans (Wang et al., 2020). COVID-19 patients frequently show higher levels of inflammation-related markers, including Interleukin-6 (IL-6), C-reactive protein (CRP), and ferritin (N. Chen et al., 2020; Zeng et al., 2020). However, the exact roles of these inflammatory markers in determining disease severity are still not fully understood (De Socio et al., 2021). Hence, this cross-sectional study sought to establish the level of treatment and determination of blood inflammation indicators for mild, moderate, severe, and critical patient in order to easily identify patient who are likely to develop severe or critical illness.

Methodology

Ethics approval:

Ethical clearance for this study was obtained from the Ethical Committee of Jagannath Gupta Institute of Medical Sciences and Hospital (JIMSH), Kolkata-700137, on November 22, 2021 under approval number JIMSH-IEC-11-2021.

Patients

In this study, conducted retrospectively, data was collected on laboratory investigations of all COVID-19 positive patients conformed by RT-qPCR (Haque et al., 2025) who had peripheral blood cytokine test done at JIMSH, Budge Budge, Kolkata, West Bengal, India within August 4, 2020 to January 11, 2022. Details of patients' information, including disease severity, age, and gender, were collected, along with markers of inflammation and complete blood counts, such as IL-6 and CRP levels. The symptoms of COVID-19 can be categorized into different risk groups like Mild, Moderate, Severe, and Critical. **Asymptomatic or Mild Symptoms (Low risk**

group): Some people infected with a COVID-19 virus may show no signs at all or may be a mild case so that it does not cause severe respiratory illness or lead to hospitalization (Zou et al., 2020). Common mild symptoms may include: Fever or chills, Cough, Muscle or body aches or Fatigue, Headache, Sore throat, Loss of taste or smell, Congestion or runny nose, Nausea or vomiting or Diarrhea. Moderate Symptoms (Intermediate risk group): There are some people who may get mild disease which may warrant the attention of a doctor but does not make them extremely sick or get admitted. **Moderate symptoms (Intermediate risk group)**: Some symptoms include: difficulty breathing; chest pain or pressure; marked fever; continuous cough; deterioration of symptoms over time. **Severe symptoms (High risk group):** Certain patients with COVID -19 may present severe symptoms that require admission to the hospital and critical care (Gong et al., 2020). Severe symptoms may include Severe difficulty in breathing or breathing rate greater or equal to 30 breaths/minute, Chest pain or pressure continuing and worsening, Inability to stay conscious or confused, Cyanosis (bluish discolouration) of the lips or face. **Critical Symptoms (Very High risk group):** Some patients will develop critical illness, with severe respiratory complications or multi-organ dysfunction (*WHO, 2021*,). Critical symptoms may include: Acute lung injury, Septic Sepsis, Kidney or liver dysfunction, Clotting abnormalities and Heart failure.

Biomarkers

Both blood samples and swab samples for biochemistry, haematology, serology and molecular biological tests were analysed at the central laboratory of JIMS Hospital, Kolkata. The level of Interleukin-6(IL-6), and D-dimer was estimated by SD Biosensor F200 and the concentration of C-reactive protein (CRP) and Lactate Dehydrogenase (LDH) was estimated by Erba XL640 Biochemical Analyzer after following the manufacturing specifications. The level of ferritin was analysed by fully automated chemiluminescence immunoassay analyzers (Mindray CL-900i). Complete blood count including White Blood Cell (WBC) was determined by the Hematological Analyzer Erba Elite 580. COVID-19 testing was performed by using the Bio-Rad CFX Connect Real-Time PCR System, which detects viral RNA, providing precise and dependable results for diagnosing SARS-CoV-2 infection.

Statistical analysis:

We analyzed all statistical data using the IBM SPSS ver-22, and for normally distributed data (such as CRP, IL-6, LDH, D-dimer, ferritin, and WBC), comparisons among the mild, Intermediate, severe, critical groups or risk factors associated with COVID-19 mortality were analysed using ANOVA and unilabiate regression analyses. Parametric test (Single Sample T- Test), used to calculate median age of covid-19 patients. For correlation analysis Spearman correlation coefficient was calculated. An unconditional logistic regression model was employed to identify associated factors for critical illness. The optimal cut off value of inflammatory parameters for critical patients, balancing sensitivity and specificity, was derived from the ROC curve using Youden's index. In order to evaluate the prediction of disease Severity and Outcomes in COVID-19 Patients we used the area under the curve analysis of ROC. Statistical analyses, including ROC curve analysis and determination of criterion values, were conducted using MedCalc Statistical Software (https:www. medcalc. org/calc/diagnostic_test. php (MedCalc for Windows, Ver. 22. 023; accessed April 24, 2024). A significance level of $p < 0.05$ was considered statistically significant.

Results

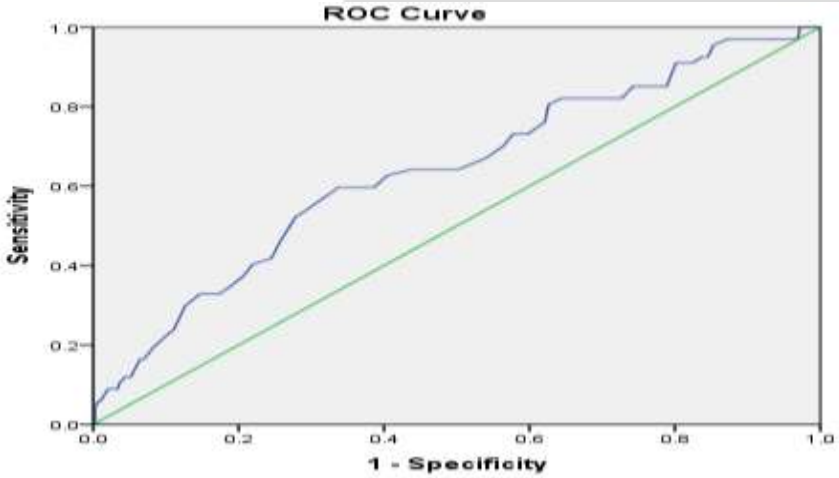
Among the 455 patients, 194 patients belonged to mild group, 194 to intermediate group, 35 to severe and 32 to critical group. In parametric test (Single Sample T- Test), median age was approximately 55.0 (54.0–57.0) $p<0.001$ and 281 (61.8%) patients were male. The median age was significant Single Sample T- Test (Table-1). There were multiple variations in laboratory results observed between the mild and moderate group compared to the severe and critical group, involving parameters such as lactate dehydrogenase (LDH), D-dimer, C-reactive protein (CRP), IL-6, ferritin, and white blood cell count (WBC) (table-1).

Table-1: Demography and some clinical parameters of COVID-19 patients admitted to JIMSH, Kolkata-137.

Variables	Total n=455	Survival n=388	Non-survival n=67	p-value
Sex, Female	174(38.24%)	154(39.69%)	20(29.85%)	<0.001
Age, year	55(53-57)	54(52-55)	62(57-66)	<0.001
D-Dimer (>500 ng/mL)				<0.001
NO	305	292	13	
YES	150	96	54	
LDH (>400units/L)				<0.001
NO	105	94	11	
YES	350	294	56	
Ferritin (> 400ng/mL)				<0.001
NO	322	288	34	
YES	133	100	33	
IL-6 (10 pg/mL)				<0.001
NO	320	297	23	
YES	135	91	44	
CRP (>6 mg/dL)				0.553
NO	219	192	27	
YES	236	196	40	
WBC (<4000 C/ µL)				<0.001
NO	352	303	49	
YES	103	85	18	

Data are presented as n (%), WBC=white blood cell count, CRP= C-reactive protein, LDH= Lactate dehydrogenase.

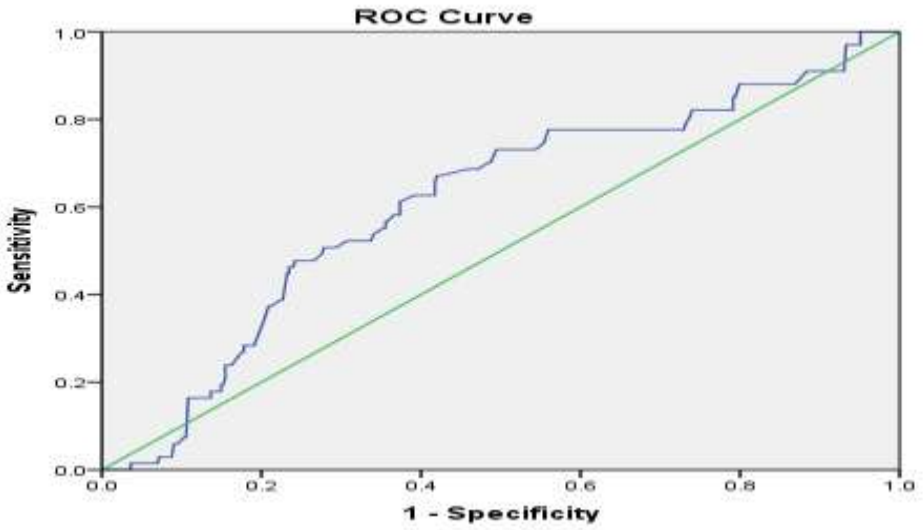
The ROC curve of IL-6 was generated (AUC: 0.611 [0.538–0.684], p=0.04), and the best cut-off point of IL-6 for mortality was 20.25 years with a specificity of 82.11% and a sensitivity of 84.21% (Figure-2).



Sen-59.70 %
Spe-66.24 %
PPV-23.39%
NPV-90.49%

Figure-1: ROC curve of age vs mortality

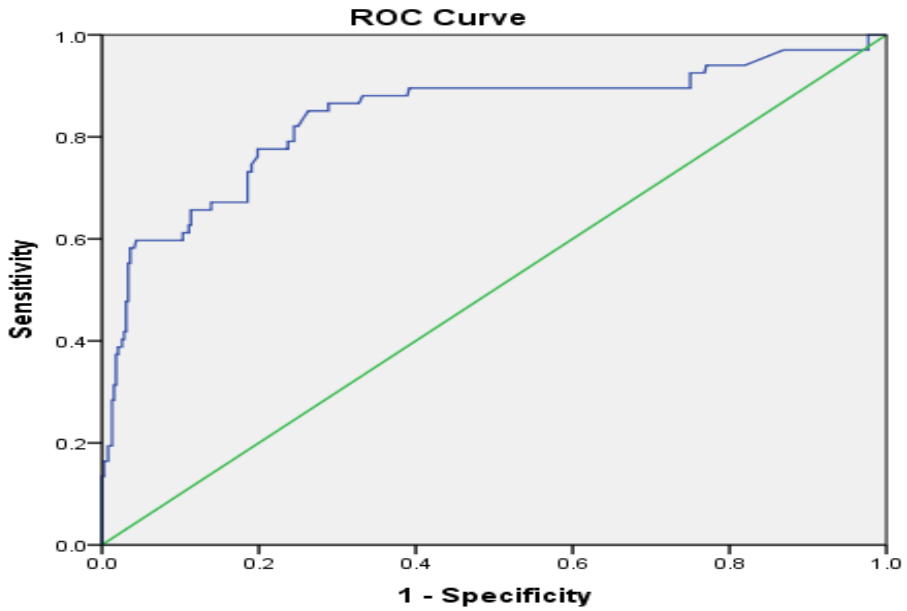
Optimal cut off value: 60.50
AUC: 0.637 (0.564–0.711), <p=0.01



Sen-84.21 %
Spe-82.11 %
PPV-65.31%
NPV-92.86%

Figure-2: ROC curve of IL-6 vs mortality

Optimal cut off value: 20.25
AUC: 0.611 (0.538–0.684), p=0.04



Sen-87.96 %
Spe-84.15 %
PPV-63.33%
NPV-95.74%

Figure-3: ROC curve of D-dimer vs mortality

Optimal cut off value: 1494
AUC: 0.980 (0.777–0.902), <p=0.01

[AUC, area under the curve; Sen, sensitivity; Spe, specificity; PPV, positive predictive value; NPV, negative predictive value.]

The D-dimer ROC curve was generated, yielding an AUC of 0.980 [0.777–0.902], with a significance level of p<0.01. Moreover, the optimal D-dimer threshold for mortality was determined to be 1494, achieving a specificity of 84.15% and a sensitivity of 87.96% (Figure-3).

The ROC curve of age was generated (AUC: 0.637 [0.564–0.711], <p=0.01), and the best cut-off point of age for mortality was 60.50 years with a specificity of 66.24% and a sensitivity of 59.70% (Figure-1).

In relation to CRP, LDH, Ferritin, and WBC levels, no significant correlation was observed with COVID-19 mortality and ROC curve, hence the supporting data for this finding were not provided.

Table-2: The distribution of patients across risk categories such as mild, moderate, severe, and critical varied.

Risk Group	Total number of patients	%	Number of healthy patients	% of healthy pt	Number of Deaths patients	% of expired pt
Low	228	50.11	225	57.99	3	4.48
Intermediate	150	32.97	138	35.57	12	17.91
High	50	10.99	24	6.19	26	38.81
Very High	27	5.93	1	0.26	26	38.81
Total	**455**	100	**388**	100	**67**	100

Pt, patient; %, percentage;

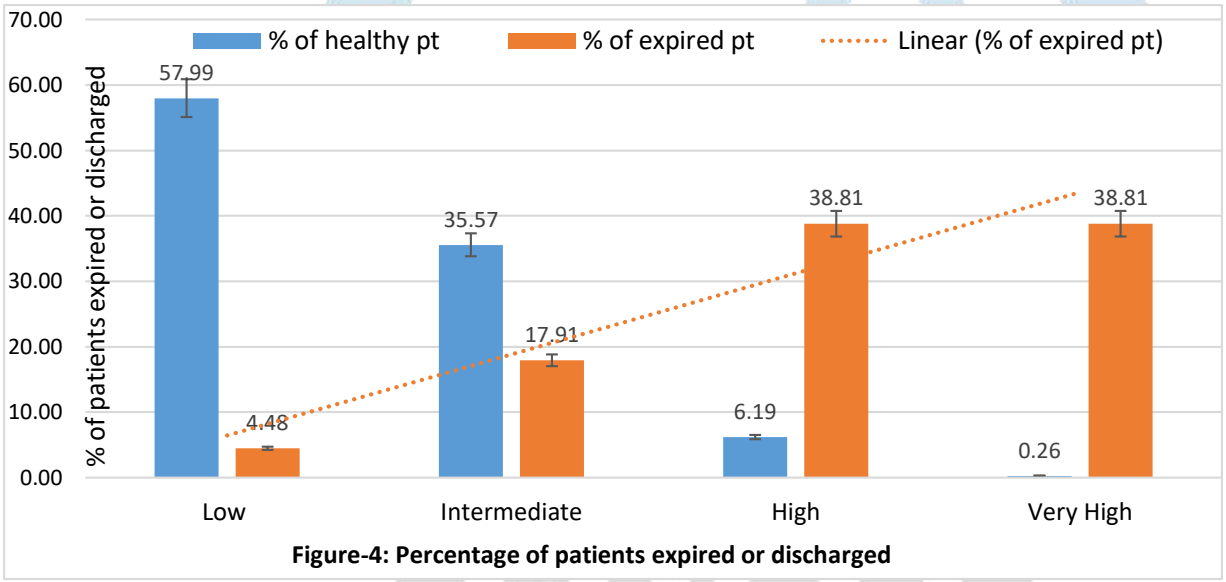


Figure-4: Percentage of patients expired or discharged

BC, LDH, D-dimer, CRP, ferritin, and IL-6 are graphically depicted below, showing their distribution between the survival and non-survival groups.

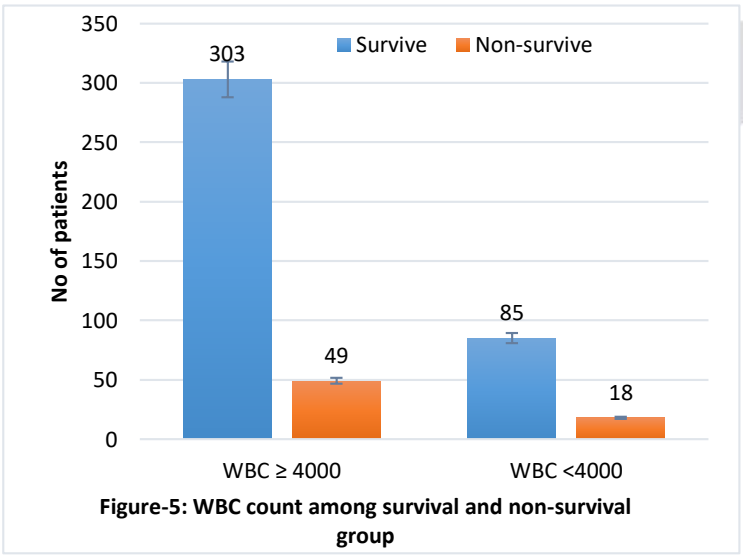


Figure-5: WBC count among survival and non-survival group

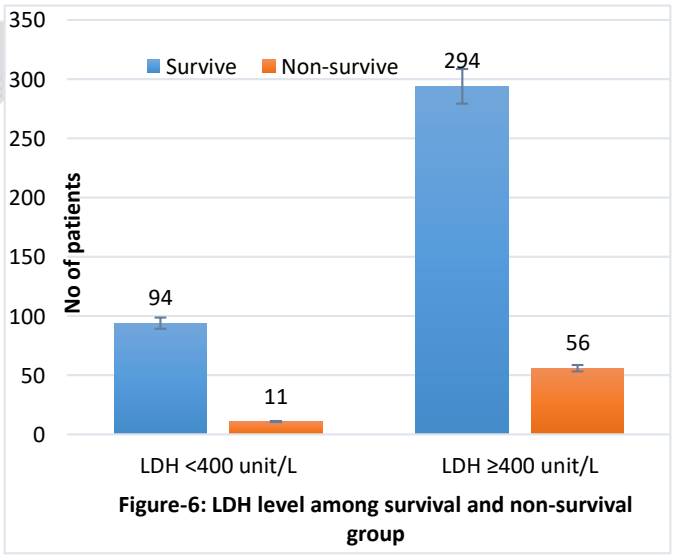


Figure-6: LDH level among survival and non-survival group

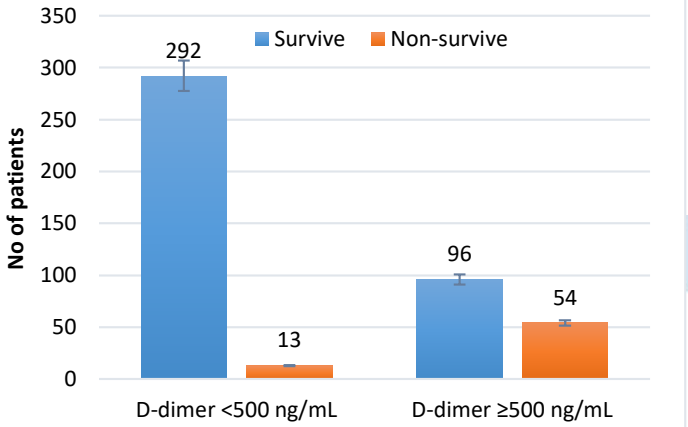


Figure-7: D-dimer level among survival and non-survival group

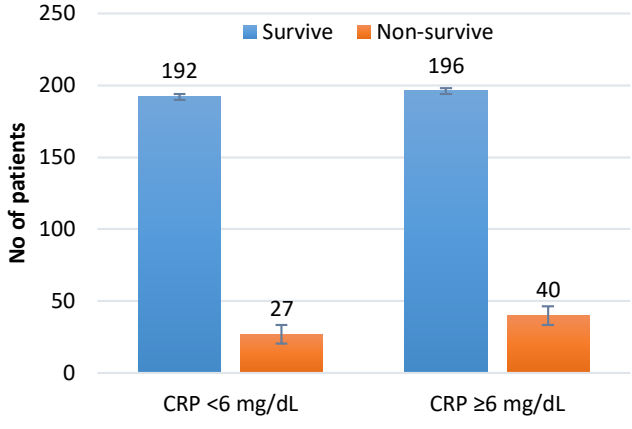


Figure-8: CRP level among survival and non-survival group

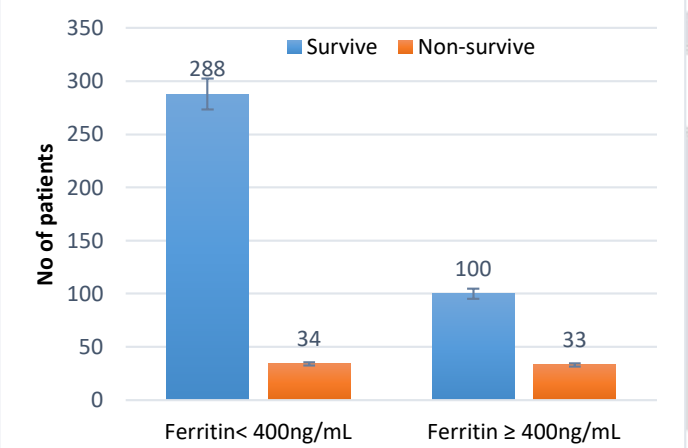


Figure-9: Ferritin value with survival and non-survival group

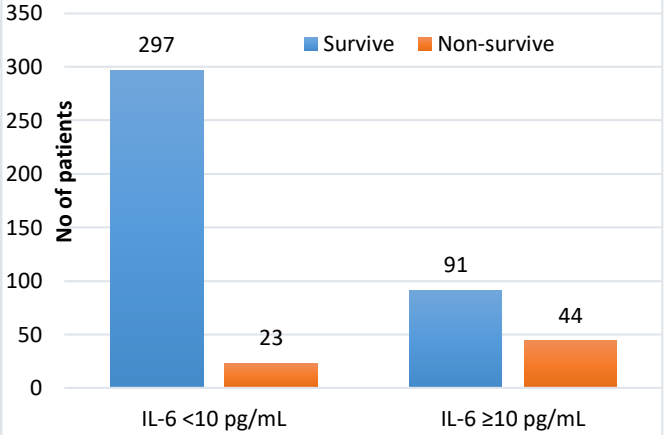


Figure-10: IL-6 value with survival and non-survival group

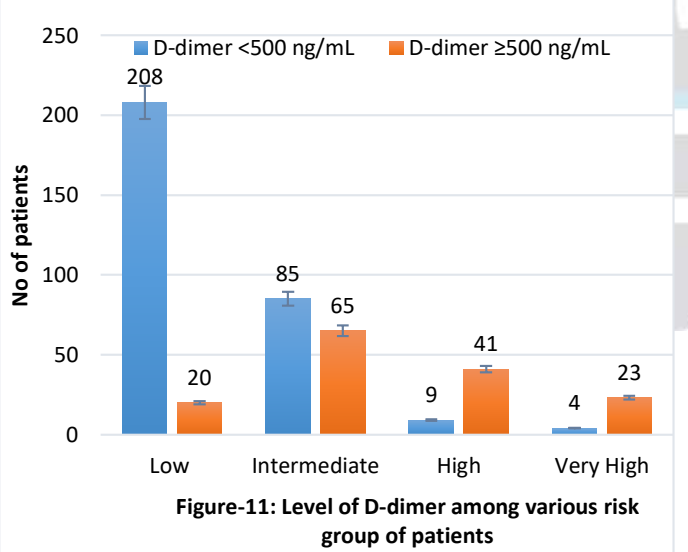


Figure-11: Level of D-dimer among various risk group of patients

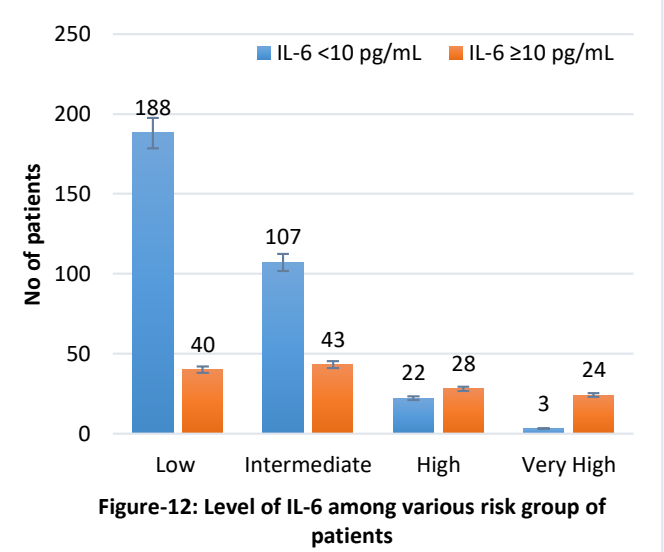


Figure-12: Level of IL-6 among various risk group of patients

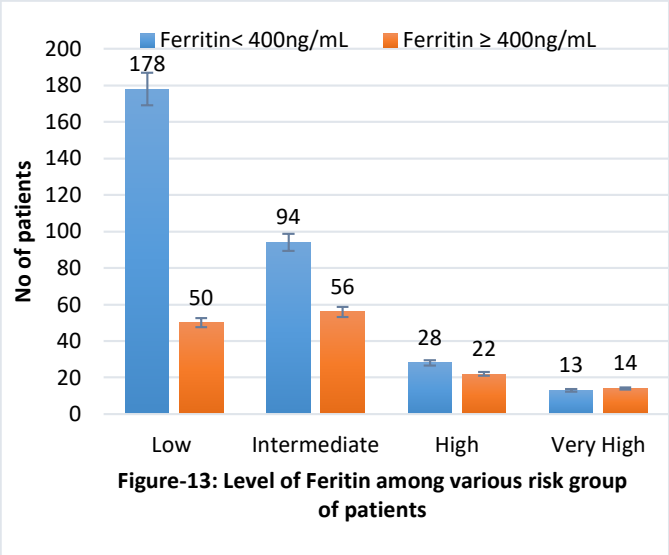


Figure-13: Level of Feritin among various risk group of patients

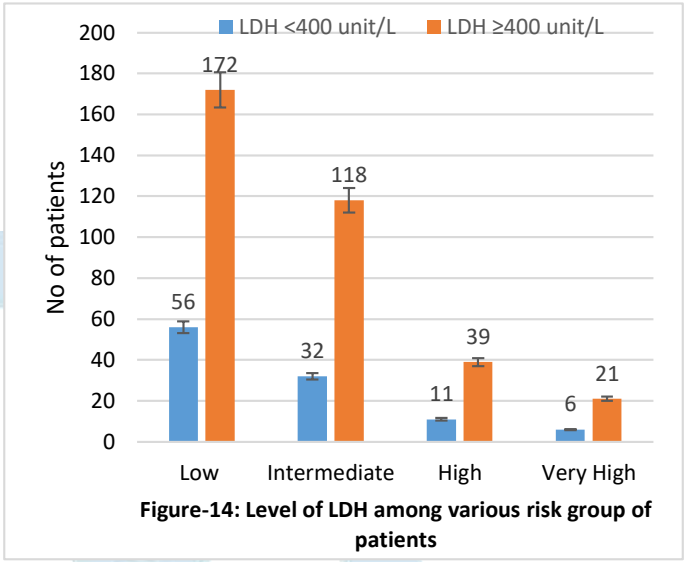


Figure-14: Level of LDH among various risk group of patients

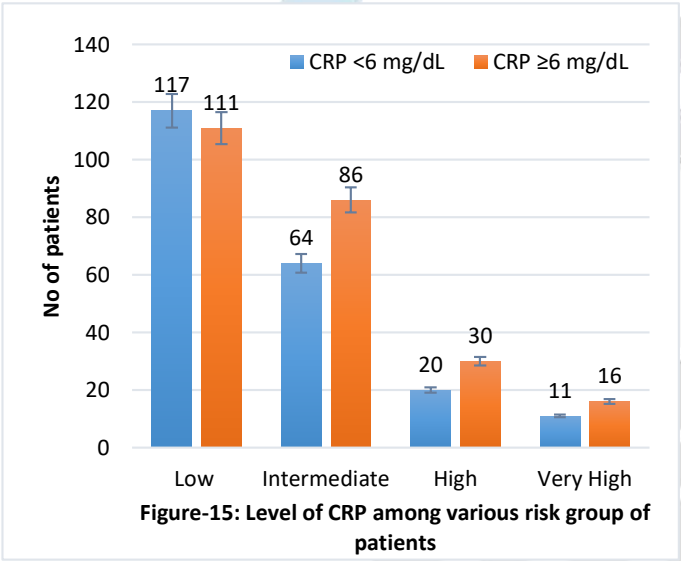


Figure-15: Level of CRP among various risk group of patients

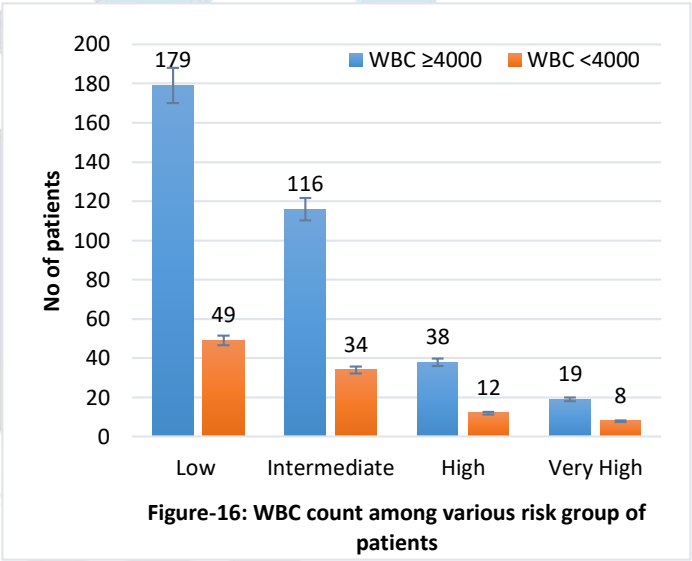


Figure-16: WBC count among various risk group of patients

Biomarkers for both survival and non-survival groups

In this comprehensive study, we examined several key biomarkers to elucidate their associations with survival outcomes in a cohort of 455 patients.

White Blood Cell (WBC) Counts: out of the 388 survivors, 303 (78.09%) had WBC counts ≥ 4000, while 85 (21.91%) had WBC counts lower than 4000. In comparison, out of 67 expired (non-survival) patients, 49 (73.13%) had WBC counts ≥ 4000, while 18 (26.87%) had WBC counts lower than 4000. This implies that more than the expected sample percentage of the surviving patients had high WBC counts suggesting that the immune system activity was associated with increased survival.

Lactate Dehydrogenase (LDH) Levels: Among the 388 survivors, 94 (24.23%) had LDH levels <400 units/L, while 294 (75.77%) had LDH level ≥ 400 units/L. In contrast, among the 67 non-survivors, only 11 (16.42%) had LDH levels < 400 units/L, while 56 (83.58%) had LHD levels ≥ 400 units/L. These results suggest that there are better survival performances among patients with low levels of LDH, making LDH as one of the prognostic biomarkers.

D-dimer Levels: Among survivors, 292 (75.26%) had D-dimer levels < 500 ng/mL, whereas 96 (24.74%) had levels above this threshold. Conversely, among non-survivors, only 13 (19.40%) had D-dimer levels < 500 ng/mL, while 54 (80.60%) had levels above 500 ng/mL. . Based on these findings, it can be concluded that IVH with D-dimer level of 500 ng/mL or higher is independently related with higher mortality risk, which points to its promising use as a biomarker in clinical practice.

C-reactive Protein (CRP) Levels: Among survivors, 192 (49.48%) had CRP levels < 6 mg/dL, and 196 (50.52%) had ≥ 6 mg/dL. In contrast, among non-survivors, 27 (40.30%) had CRP levels below 6 mg/dL, while 40 (59.70%) had ≥ 6 mg/dL. This points to the fact that increased CRP levels in the community, CRP ≥ 6 mg/dL could be associated with higher mortality risk and, hence, could be used as a biomarker to adverse outcomes.

Ferritin Levels: Ferritin levels were more roughly equally split between the survivor and non-survivor groups. Among survivors, 288 (74.23%) had ferritin levels below 400 ng/mL, whereas among non-survivors, 34 (50.75%) had levels below this threshold. Conversely, 100 (25.77%) of survivors and 33 (49.25%) of non-survivors had ferritin levels above 400 ng/mL. These findings propose a differential relation in which whatever, higher ferritin (≥400 ng/mL) is not always indicative of poor survival and needs further evaluation.

Interleukin-6 (IL-6) Levels: Among survivors, 297 (76.55%) had IL-6 levels below 10 pg/mL, as compared to 91 (23.45%) with ≥ 10 pg/mL. In contrast, among non-survivors, only 23 (34.33%) had IL-6 levels below 10 pg/mL, while 44 (65.67%) had ≥ 10 pg/mL. These findings suggest that the use of IL-6 moving up with mortality threat level is more than 10 pg/mL, making IL-6 perhaps a tremendous biomarker for threat estimate.

Biomarkers in the risk group

In our study 455 patients selected, we carefully analysed the distribution of the biomarker levels across different risk group. Among the 228 patients categorized in the low-risk group, a significant majority, 208 (91.23%), showed D-dimer levels < 500 ng/mL, while 20 (8.77%) had D-dimer levels ≥ 500 ng/mL. Regarding the intermediate risk with 150 patients, 85 (56.67%) had D-dimer levels < 500 ng/mL, while 65 (43.33%) had D-dimer levels ≥ 500 ng/mL. Similarly, within the high-risk group of 50 patients, 9 (18.00%) had D-dimer levels < 500 ng/mL, while 41 (82.00%) had D-dimer levels ≥ 500 ng/mL. In the very high-risk group consisting of 27 patients, 4 (14.81%) had D-dimer levels < 500 ng/mL, while 23 (85.19%) had D-dimer levels ≥ 500 ng/mL. These ground also emphasise the significant relationship between the hike in D-dimer levels and high risk categorisation which can infer that D-dimer maybe a useful biomarker for risk assessment in real practice settings. These results imply had-linearity relationship between D-dimer level and higher risk categorization and D-dimer could be used as potential diagnostic biomarker in clinic.

We analysed interleukin-6 (IL-6) levels across the same risk groups. Among the 228 patients categorized in the low-risk group, 188 (82.46%) had IL-6 levels < 10 pg/mL, while 40 (17.54%) had IL-6 levels ≥10 pg/mL. In the intermediate-risk group comprising 150 patients, 107 (71.33%) exhibited IL-6 levels <10 pg/mL, compared to 43 (28.67%) showed IL-6 levels ≥10 pg/mL. Comparably, within the high-risk group of 50 patients, 22 (44.00%) had IL-6 levels <10 pg/mL, while 28 (56.00%) had IL-6 levels ≥10 pg/mL. Among the 27 patients in the very high-risk group, 3 (11.11%) had IL-6 levels < 10 pg/mL, while 24 (88.89%) had IL-6 levels ≥10 pg/mL. These results show a trend toward higher IL-6 levels in groups with higher risk, suggesting that IL-6 may be used as a biomarker for risk classification in clinical settings.

Our study explored ferritin levels across these risk groups. Among the 228 patients categorized in the low-risk group, 178 (78.07%) had ferritin levels < 400 ng/mL, while 50 (21.93%) had ferritin levels ≥ 400 ng/mL. In the intermediate-risk group comprising 150 patients, 94 (62.67%) exhibited ferritin levels < 400 ng/mL, compared to 56 (37.33%) patients ferritin levels ≥ 400 ng/mL, within the high-risk group of 50 patients, 28 (56.00%) had ferritin levels < 400 ng/mL, while 22 (44.00%) had ferritin levels ≥ 400 ng/mL. In the very high-risk group consisting of 27 patients, 13 (48.15%) had ferritin levels < 400 ng/mL, while 14 (51.85%) had ferritin levels ≥ 400 ng/mL. Cumulatively these findings depict a clear indication of increasing ferritin levels with higher risk categories, hence the utility of ferritin as a biomarker of risk stratification in clinics.

Our study explored LDH levels across these risk groups. Among the 228 patients categorized in the low-risk group, 56 (24.56%) had LDH levels < 400 ng/mL, while 172 (75.44%) had LDH levels ≥ 400 ng/mL. In the

intermediate-risk group comprising 150 patients, 32 (21.33%) exhibited LDH levels < 400 ng/mL, compared to 118 (78.67%) patients LDH levels ≥ 400 ng/mL, within the high-risk group of 50 patients, 11 (22.00%) had LDH levels < 400 ng/mL, while 39 (78.00%) had LDH levels ≥ 400 ng/mL. In the very high-risk group consisting of 27 patients, 6 (22.22%) had LDH levels < 400 ng/mL, while 21 (77.78%) had LDH levels ≥ 400 ng/mL. It is evident from the above results that there is a trend towards increased LDH across the higher risk groups indicating LDH could be useful in risk assessment in clinic practice.

C-reactive protein (CRP) has been used in our study with regards to the highlighted risk groups. Of the 228 patients in the low risk group, 117 (51%) showed CRP level < 6 mg/dL while 111 (48.68%) had elevated CRP levels ≥ 6 mg/dL. In the intermediate risk group of 150 patients, 64 (42. 67%) patients had CRP levels <6 mg/dl while 86 (57.33%) had ≥6mg/dl. Likewise, within the high-risk group of 50 patients, 20 (40.00%) had CRP levels below 6 mg/dL, while 30 (60.00%) had CRP levels ≥ 6 mg/dL. In the very high-risk group consisting of 27 patients, 11 (40.74%) had CRP levels below 6 mg/dL, with 16 (59.26%) had CRP levels ≥ 6 mg/dL. The results presented here are in line with a trend, which observed elevated CRP levels in subjects belonging to higher risk groups, and may indicate that additional investigation of the needed cut-off points for using CRP as a risk biomarker in clinical practice is indeed warranted.

The relationship between risk group and white blood cell (WBC) counts is what we aimed to investigate through a study that involved 455 patients. The findings reveal that out of the 228 patients comprising the low risk group, 179 (78.51%) had WBC counts ≥ 4000 while 49 (21.49%) had WBC counts > 4000. In the intermediate risk group of 150 patients,116 (77.33%) exhibited WBCs levels≥4000 compared to 34 (22.67%) whose WBC levels were <4000. Within the high-risk group of 50 patients, 38(76%) had WBC counts ≥ 4000, while 12 (24%) had WBC counts < 4000. In the very high-risk group consisting of 27 patients, 19 (70.37%) had WBC counts ≥ 4000, while 8 (29.63%) had WBC counts < 4000. From these findings, it is evident that elevated WBC count is linked with higher risk categorization; therefore, WBC potentiation as a biomarker for risk in clinical contexts.

Discussion

On the analysis of our research outcomes involving 455 patients who were devided into different risks groups. The low-risk group, comprising 228 patients (50.11% of the total), demonstrated a notably high percentage of healthy individuals (57.99%) and a low mortality rate (4.48%). This indicates that generally, patients in this category were more likely to experience good outcomes with less chance for adverse events.

In contrast, the intermediate-risk group composed of 150 persons (32.97%), showed a lower proportion of healthy individuals 35.57% and thus a greater mortality rate 17.91% implying moderate level of risk which calls for close observation and intervention measures.

Emphasizing the increased susceptibility of patients in this category, the high-risk group, which consisted of 50 patients (10.99%), showed an even lower percentage of healthy patients (6.19%) and a significantly higher fatality rate (38.81%). The very high-risk group, which consisted of 27 patients (5.93%), also had a very high mortality rate of 38.81% and a very low percentage of healthy patients (0.26%), underscoring the severe nature of their disease and the urgent need for intense medical care. These results highlight the success of the risk stratification approach in forecasting patient outcomes and informing clinical management strategies, which can enhance care delivery and boost survival rates across various risk groups. (Table-2).

In a retrospective cohort study(F. Zhou et al., 2020) involving 191 adult inpatients with laboratory-confirmed COVID-19, multivariable regression analysis revealed a higher likelihood of in-hospital mortality linked to older age (odds ratio 1.10, 95% CI 1.03–1.17, per year increase; p = 0.0043) and D-dimer levels exceeding 1 μg/mL upon admission (18.42, 2.64–128.55; p = 0.0033).

Risk factors linked to the onset of acute respiratory distress syndrome (ARDS) and the progression from ARDS to mortality in COVID-19 patients comprised older age(hazard ratio [HR], 3.26; 95% CI 2.08–5.11; and HR,

6.17; 95% CI, 3.26–11.67, respectively), elevated levels of lactate dehydrogenase (LDH) (HR, 1.61; 95% CI, 1.44–1.79; and HR, 1.30; 95% CI, 1.11–1.52, respectively), and increased D-dimer levels (HR, 1.03; 95% CI, 1.01–1.04; and HR, 1.02; 95% CI, 1.01–1.04, respectively)(Wu et al., 2020).

According to Zhang et al. (2020) and Ferrari et al. (2020), older males (60 years of age and older) who have other underlying medical disorders are frequently linked to higher disease severity, progression, and unfavourable outcomes. The present study found that age and D-dimer levels were significant predictors of COVID-19 mortality. Older patients with D-dimer levels >1494 ng/mL had an increased risk of death with age being ≥ 60.50 years thus requiring close monitoring to these older people and those having high D-dimer levels.
An IL-6 ROC curve analysis was performed which revealed an AUC of 0.611 (0.538-0.684) at significant level p=0.04, thus optimal IL-6 cut-off for mortality prediction is 20.25 giving specificity 82.11 % and sensitivity 84.21 %. Similarly, the ROC curve for D-dimer revealed an AUC of 0.980 (0.777–0.902) with a p-value less than 0.01. The best threshold for D-dimer to predict mortality was determined to be 1494, with specificity and sensitivity values of 84.15% and 87.96%, respectively. Furthermore, the age-based ROC analysis showed an AUC of 0.637 (0.564–0.711), with $p < 0.01$. The optimal age threshold for predicting mortality was found to be 60.5 years, offering a specificity of 66.24% and a sensitivity of 59.70%.

The differences observed in the inflammatory biomarkers across severity categories suggest the potential role of these biomarkers in disease progression. (Zhou et al., 2020; Zhang et al., 2020; Chen et al., 2020). Most interestingly, the two biomarkers IL-6 and D-dimer were significantly increased in the non-survival group, which means that both could be used to predict poor prognosis.

Nonetheless, the poor correlation of CRP, LDH, Ferritin, WBC with COVID mortality casts doubt about these biomarkers as an isolated risk predictors. It is, therefore, necessary to carry further studies to understand how they contribute in disease progression and prognosis.

Conclusion

This large cross-sectional study examines the association of inflammatory biomarkers with survival among 455 patients with COVID-19. The analysis presents strong correlations of biomarker concentrations with mortality odds ratios. This means that; the high WBC count among the survivors may be an indicator of a good immunity response that will lead to survival. On the other hand, higher serum Lactate Dehydrogenase (LDH), D-dimer, C-reactive Protein (CRP), and Interleukin-6(IL-6) levels rated higher on the odds ratio to mortality, suggesting their value as prognosticators. Further stratification of the data by risk groups also reaffirmed the observed patterns of biomarkers relate to the respective risk levels, thereby confirming their relevance to risk evaluation and gradation among patients. Thus, the results of the current study add insight to the clinical practice in the management of COVID-19 patients and support the regulation of the described biomarkers in order to predict clinically significant outcomes and enhance patient prognosis. More studies should be done to confirm such findings and identify other biomarkers to enhance predictive capacity of risk evaluation in varied patients. The cross-sectional study design, the retrospective nature of the survey and the fact that it was carried among the patients of a single center may pose some questions to the external validity of the findings. More work that involved larger number of patients, preferably involving multiple centres, is required to confirm the findings and to assess further other possible biomarkers

Reference

1. Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., Zhang, L., Fan, G., Xu, J., Gu, X., Cheng, Z., Yu, T., Xia, J., Wei, Y., Wu, W., Xie, X., Yin, W., Li, H., Liu, M., Xiao, Y., … Cao, B. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet (London, England)*, *395*(10223), 497–506. https://doi.org/10.1016/S0140-6736(20)30183-5.
2. Chen, X., Zhao, B., Qu, Y., Chen, Y., Xiong, J., Feng, Y., Men, D., Huang, Q., Liu, Y., Yang, B., Ding, J., & Li, F. (2020). Detectable Serum Severe Acute Respiratory Syndrome Coronavirus 2 Viral Load (RNAemia) Is Closely Correlated With Drastically Elevated Interleukin 6 Level in Critically Ill Patients With Coronavirus

Disease 2019. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*, *71*(8), 1937–1942. https://doi.org/10.1093/cid/ciaa449.

3. Li, Q., Guan, X., Wu, P., Wang, X., Zhou, L., Tong, Y., Ren, R., Leung, K. S. M., Lau, E. H. Y., Wong, J. Y., Xing, X., Xiang, N., Wu, Y., Li, C., Chen, Q., Li, D., Liu, T., Zhao, J., Liu, M., Tu, W., … Feng, Z. (2020). Early Transmission Dynamics in Wuhan, China, of Novel Coronavirus-Infected Pneumonia. *The New England journal of medicine*, *382*(13), 1199–1207. https://doi.org/10.1056/NEJMoa2001316.
4. Phelan, A. L., Katz, R., & Gostin, L. O. (2020). The Novel Coronavirus Originating in Wuhan, China: Challenges for Global Health Governance. *JAMA*, *323*(8), 709–710. https://doi.org/10.1001/jama.2020.1097.
5. Graham, R. L., & Baric, R. S. (2020). SARS-CoV-2: Combating Coronavirus Emergence. *Immunity*, *52*(5), 734–736. https://doi.org/10.1016/j.immuni.2020.04.016.
6. Zhou, F., Yu, T., Du, R., Fan, G., Liu, Y., Liu, Z., Xiang, J., Wang, Y., Song, B., Gu, X., Guan, L., Wei, Y., Li, H., Wu, X., Xu, J., Tu, S., Zhang, Y., Chen, H., & Cao, B. (2020). Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet (London, England)*, *395*(10229), 1054–1062. https://doi.org/10.1016/S0140-6736(20)30566-3.
7. Wang, D., Hu, B., Hu, C., Zhu, F., Liu, X., Zhang, J., Wang, B., Xiang, H., Cheng, Z., Xiong, Y., Zhao, Y., Li, Y., Wang, X., & Peng, Z. (2020). Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. *JAMA*, *323*(11), 1061–1069. https://doi.org/10.1001/jama.2020.1585.
8. Chen, N., Zhou, M., Dong, X., Qu, J., Gong, F., Han, Y., Qiu, Y., Wang, J., Liu, Y., Wei, Y., Xia, J., Yu, T., Zhang, X., & Zhang, L. (2020). Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet (London, England)*, *395*(10223), 507–513. https://doi.org/10.1016/S0140-6736(20)30211-7.
9. Zeng, F., Huang, Y., Guo, Y., Yin, M., Chen, X., Xiao, L., & Deng, G. (2020). Association of inflammatory markers with the severity of COVID-19: A meta-analysis. *International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases*, *96*, 467–474. https://doi.org/10.1016/j.ijid.2020.05.055.
10. De Socio, G. V., Gidari, A., Sicari, F., Palumbo, M., & Francisci, D. (2021). National Early Warning Score 2 (NEWS2) better predicts critical Coronavirus Disease 2019 (COVID-19) illness than COVID-GRAM, a multi-centre study. *Infection*, *49*(5), 1033–1038. https://doi.org/10.1007/s15010-021-01620-x.
11. Zou, L., Ruan, F., Huang, M., Liang, L., Huang, H., Hong, Z., Yu, J., Kang, M., Song, Y., Xia, J., Guo, Q., Song, T., He, J., Yen, H. L., Peiris, M., & Wu, J. (2020). SARS-CoV-2 Viral Load in Upper Respiratory Specimens of Infected Patients. *The New England journal of medicine*, *382*(12), 1177–1179. https://doi.org/10.1056/NEJMc2001737.
12. Gong, J., Dong, H., Xia, Q. S., Huang, Z. Y., Wang, D. K., Zhao, Y., Liu, W. H., Tu, S. H., Zhang, M. M., Wang, Q., & Lu, F. E. (2020). Correlation analysis between disease severity and inflammation-related parameters in patients with COVID-19: a retrospective study. *BMC infectious diseases*, *20*(1), 963. https://doi.org/10.1186/s12879-020-05681-5.
13. WHO, COVID-19 Treatment Guidelines Panel. *Coronavirus Disease 2019 (COVID-19) Treatment Guidelines*. National Institutes of Health; [23 October 2021]. Available at https://www.covid19treatmentguidelines.nih.gov/overview/clinical-spectrum/.
14. Wu, C., Chen, X., Cai, Y., Xia, J., Zhou, X., Xu, S., Huang, H., Zhang, L., Zhou, X., Du, C., Zhang, Y., Song, J., Wang, S., Chao, Y., Yang, Z., Xu, J., Zhou, X., Chen, D., Xiong, W., Xu, L., … Song, Y. (2020). Risk Factors Associated With Acute Respiratory Distress Syndrome and Death in Patients With Coronavirus Disease 2019 Pneumonia in Wuhan, China. *JAMA internal medicine*, *180*(7), 934–943. https://doi.org/10.1001/jamainternmed.2020.0994.
15. Zhang, B., Zhou, X., Qiu, Y., Song, Y., Feng, F., Feng, J., Song, Q., Jia, Q., & Wang, J. (2020). (Zhang et al., 2020)Clinical characteristics of 82 cases of death from COVID-19. *PloS one*, *15*(7), e0235458. https://doi.org/10.1371/journal.pone.0235458.

16. Ferrari, D., Motta, A., Strollo, M., Banfi, G., & Locatelli, M. (2020). Routine blood tests as a potential diagnostic tool for COVID-19. *Clinical chemistry and laboratory medicine*, *58*(7), 1095–1099. https://doi.org/10.1515/cclm-2020-0398.
17. Zhou, Y., Han, T., Chen, J., Hou, C., Hua, L., He, S., Guo, Y., Zhang, S., Wang, Y., Yuan, J., Zhao, C., Zhang, J., Jia, Q., Zuo, X., Li, J., Wang, L., Cao, Q., & Jia, E. (2020). Clinical and Autoimmune Characteristics of Severe and Critical Cases of COVID-19. *Clinical and translational science*, *13*(6), 1077–1086. https://doi.org/10.1111/cts.12805.
18. Chen, R., Sang, L., Jiang, M., Yang, Z., Jia, N., Fu, W., Xie, J., Guan, W., Liang, W., Ni, Z., Hu, Y., Liu, L., Shan, H., Lei, C., Peng, Y., Wei, L., Liu, Y., Hu, Y., Peng, P., Wang, J., … Medical Treatment Expert Group for COVID-19 (2020). Longitudinal hematologic and immunologic variations associated with the progression of COVID-19 patients in China. The Journal of allergy and clinical immunology, 146(1), 89–100. https://doi.org/10.1016/j.jaci.2020.05.003.
19. Haque, S. K. E., Bhadra, S., & Pal, N. K. (2025). OPTIMIZED PRIMER & PROBE DESIGN FOR TARGETING THE RAPIDLY EVOLVING SARS-COV-2 GENOME USING NCBI AND INTEGRATED DNA TECHNOLOGY (IDT): ENSURING SPECIFICITY AND SENSITIVITY IN REAL-TIME DETECTION. In World Journal of Pharmaceutical Research, *World Journal of Pharmaceutical Research* (Vol. 14, Issue 7, pp. 873–900). https://doi.org/10.20959/wjpr20257-36016