Original Research Article

The Egyptian Journal of Immunology
Volume 29 (3), 2022: 44–53.
www.Ejimmunology.org

Inetrleukin-6 and C-reactive protein as predictors of mortality among critically ill COVID-19 patients in Assiut university hospitals (ICUs)

Asmaa O. Ahmed, Hebat Allah G. Rashed, Alshymaa F. Solyman and Doaa M. Abd El-Kareem

Department of Clinical Pathology, Faculty of Medicine, Assiut University, Assiut, Egypt.

Corresponding author: Alshymaa F. Solyman, Department of Clinical Pathology, Faculty of Medicine, Assiut University, Assiut, Egypt.
Email: alshymaafaheem@med.aun.edu.eg

Abstract

COVID-19 represents a serious global threat due to scarcity of definitive cure and its infectious nature. The death rate of COVID-19 patients admitted to hospitals was quite high, and cytokine storms could be the mechanism of severity. Interleukin-6 (IL6) and C-reactive protein (CRP) may predict severity and mortality. We attempted to determine the role of IL6 and CRP as predictors of death in intensive care unit (ICU) patients. This Cross-sectional hospital study included 100 patients admitted to ICUs at Assiut University Hospitals from October 2020 to October 2021. Data including age, sex and comorbidities were recorded, laboratory investigations included CRP, ferritin, and IL6. Data were collected and analyzed. Morality predictors in ICU patients with COVID-19 infection were older age (>60 years), presence of diabetes mellitus, chest diseases, CRP >49, IL-6 >70 pg/ml. In conclusion, early ranking and identification of people, who are at risk of death among ICU patients, by monitoring of CRP, IL6, early treatment of cytokine storm, and good management of pre-existing comorbidities would be a very useful approach to reduce the mortality among ICU patients.

Keywords: COVID-19, Predictors, Mortality, IL6, CRP.

Date received: 25 February 2022; **accepted:** 16 May 2022.

Introduction

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), represents a serious global threat due to scarcity of definitive cure and its infectious nature. The death rate of COVID-19 patients admitted to hospitals was quite high, ranging from 1-52%. While the death rate of COVID-19 patients in critical care units was extremely high, ranging from 6% to 86% of those admitted.[1]

Interleukin-6 (IL-6), which is a B-cell stimulatory factor 2, stimulates the full maturation of activated B-cells to be antibody-producing cells.[2] The widely used inflammatory biomarker C-reactive protein (CRP) is generated and released by the liver in response to IL-6 activation. When the body is in an acute inflammatory condition, CRP levels rise, and in most circumstances, they rise in proportion to the severity of the disease, then fall as the inflammation subsides. Hyper inflammatory condition could be an outcome of SARS-CoV-2

infection that can progress to cytokine storm, septic shock, coagulation abnormalities, and multiorgan failure in some patients.[3,4]

In critically ill COVID-19 patients, hyper inflammation manifests itself in the form of increased serum CRP, suggesting that disease severity may be caused by cytokine storms. COVID-19 patients' severity and prognosis appear to be predicted by increased serum IL-6 expression.[5]

In the current study, we attempted to determine the clinical characteristics of COVID-19 patients and the role of IL6 and CRP as predictors of death in ICUs patients at Assiut University Hospitals. This will be critical for the early detection of those who are at risk of death among ICU patients.

Materials and Methods

Study population and ethical statement

This Cross-sectional hospital-based study was performed in the Microbiology Unit, Clinical Pathology Department at Assiut University Hospital during the period from October 2020 to October2021. The study included 100 patients with confirmed COVID-19 and admitted to ICU. Patients with COVID-19 but did not admit to ICU were excluded. Based on the outcome of studied patients, they were subdivided into two groups either survivors (n= 60 patients) or non-survivors (n= 40 patients).

The Institutional Review Board of the Faculty of Medicine, Assiut University, reviewed and approved the study protocol (October 2020). Also, the study was registered as a clinical trial (registration Clinical Trials ID: NCT004479982).

Demographic, clinical and laboratory characteristics

Demographic data including age, sex and comorbidities were recorded. Laboratory tests as complete blood picture was done by a full automated hematology system (ADVIA®2120i hematology system, Siemens-Healthineers, GmbH Henkestr. 127 91052 Erlangen, Germany), according to the manufacturer's instructions. D-dimer and international randomized ratio (INR) were done by a full automated coagulation analyzer (Sysmex CS-5100®, Siemens-Healthineers, GmbH Henkestr. 127 91052 Erlangen, Germany) according to the manufacturer's instructions. Liver and kidney function tests, ferritin, magnesium, calcium, sodium, potassium, glucose, CRP, and ferritin were done by a full automated blood chemistry system (ADVIA® 1800 chemistry system, Siemens-Healthineers, GmbH Henkestr. 127 91052 Erlangen, Germany), according to the manufacturer's instructions. Lactate dehydrogenase (LDH) was done by a full automated chemistry system (Dimension® RxL Max® Chemistry System, Siemens-Healthineers, GmbH Henkestr. 127 91052 Erlangen, Germany), according to the manufacturer's instructions. IL6 was done by an immunoassay system (ADVIA® CentaurXPT, Siemens-Healthcare GmbH Henkestr. 127 91052 Erlangen, Germany), according to the manufacturer's instructions.

Statistical methods

Data were collected and analyzed using SPSS (Statistical Package for the Social Science, version 20, IBM, and Armonk, New York). Continuous data was expressed in form of mean ± SD while nominal data was expressed in form of frequency (percentage). Chi² test was used to compare the nominal data of different groups in the study while student t-test was used to compare mean of continuous data of groups. Accuracy of CRP and IL6 was determined by the receiver operator characteristic (ROC) curve. Logistic regression analysis was used to determine the independent risk factors for prediction of mortality among patients with COVID-19 in ICU. A *P*-value of <0.05% was considered statistically significant.

Results

Baseline data of the enrolled patients according to their outcome

The mean age (standard deviation, SD) of enrolled patients was 57.62 (14.54) years. The majority (53%) of them were males. A total of 60 (60%) patients survived the whole study period while the others were deteriorated and died (Table 1).

The mean age of non-survivors was significantly higher in comparison to survivors (61.28 ± 15.06 vs. 55.18 ± 13.78; *P*= 0.04). Also, a higher percentage of non-survivors were males (26 (65%) vs. 21 (35%); *P*< 0.001), diabetics (diabetes mellitus, DM) (33 (82.5%) vs. 26 (43.3%); *P*< 0.001) and had chest diseases (17 (42.5%) vs. 4 (6.7%); *P*< 0.001) (Table 1).

Table 1. Baseline data of the enrolled patients according to their outcome.

	Total (n= 100)	Survivors (n= 60)	Non-survivors (n= 40)	*P* value
Age (years)	57.62 ± 14.54	55.18 ± 13.78	61.28 ± 15.06	0.04
Sex				
Male	47 (47%)	21 (35%)	26 (65%)	<0.001
Female	53 (53%)	39 (65%)	14 (35%)	
Diabetes mellitus	59 (59%)	26 (43.3%)	33 (82.5%)	<0.001
Hypertension	12 (12%)	5 (8.3%)	7 (17.5%)	NS
Chest disease	21 (21%)	4 (6.7%)	17 (42.5%)	<0.001
Chronic kidney disease	4 (4%)	1 (1.7%)	3 (7.5%)	NS
Ischaemic heart disease	9 (9%)	3 (5%)	6 (15%)	NS

Data expressed as frequency (percentage), mean (±SD). *P* value > 0.05 is not significant (NS).

Baseline laboratory data of enrolled patients according to the outcome

The non-survivor group had significantly higher leucocytes, neutrophils, D-dimer, CRP, INR, ferritin, LDH aspartate transaminase, alanine transaminase, and IL-6 than the survivor group (Table 2). However, the survivor group had significantly higher lymphocytes and serum calcium than the non-survivor group (Table 2). Nevertheless, other measured parameters were not different between the two groups.

Table 2. Baseline laboratory data of enrolled patients according to the outcome.

	Total (n= 100)	Survivors (n= 60)	Non-survivors (n= 40)	*P* value
Leucocytes (10^3/μl)	14.34 ± 8.21	11.03 ± 5.98	19.31 ± 8.63	< 0.001
Neutrophil (10^3/μl)	12.26 ± 7.84	8.84 ± 5.87	17.39 ± 7.68	< 0.001
Lymphocytes (10^3/μl)	1.40 ± 0.96	1.59 ± 1	1.13 ± 0.85	0.02
Platelets (10^3/μl)	304.76 ± 140.48	302.18 ± 144.74	308.63 ± 136.50	NS
Hemoglobin (g/dl)	11.89 ± 1.91	11.87 ± 1.71	11.93 ± 2.19	NS
Ferritin (ng/ml)	1282.59 ± 583.68	905.95 ± 387.29	1847.55 ± 705.72	< 0.001
D. dimer (ng/ml)	3.54 ± 2.76	2.08 ± 1.04	5.73 ± 3.08	< 0.001
C-reactive protein (mg/dl)	60.17 ± 56.73	37.59 ± 14.56	94.05 ± 34.56	< 0.001
International randomized ratio	1.11 ± 0.22	1.06 ± 0.22	1.17 ± 0.21	0.01
Sodium (mmol/l)	140.31 ± 6.95	138.88 ± 5.60	142.45 ± 8.22	NS
Potassium (mg/dl)	4.07 ± 0.76	4 ± 0.65	4.19 ± 0.89	NS
Glucose (mmol/l)	10.81 ± 6.61	10.74 ± 6.99	10.93 ± 6.08	NS
Bilirubin (mmol/l)	9.70 ± 6.70	8.25 ± 4.91	11.89 ± 8.33	NS
Direct bilirubin (mmol/l)	3.91 ± 2.39	3.54 ± 1.35	4.46 ± 2.10	NS
Aspartate transaminase (U/l)	45.35 ± 23.41	33.69 ± 12.19	62.85 ± 23.45	0.02
Alanine transaminase (U/l)	50.12 ± 24.45	38.06 ± 13.33	68.20 ± 24.09	0.02
Alkaline phosphatase (U/l)	97.05 ± 54.56	98.78 ± 59.76	94.45 ± 46.29	NS
Proteins (mg/dl)	59.16 ± 7.29	61.19 ± 6.51	57.23 ± 7.83	NS

Table 2. (Continued).

	Total (n= 100)	Survivors (n= 60)	Non-survivors (n= 40)	*P* value
Albumin (mg/dl)	32.79 ± 7.45	33.20 ± 8.83	32.17 ± 4.73	NS
Magnesium (mg/dl)	2.11 ± 0.42	2.07 ± 0.40	2.16 ± 0.45	NS
Urea (mg/dl)	14.81 ± 4.09	14.29 ± 5.61	15.59 ± 3.45	NS
Creatinine (mmol/l)	117.52 ± 45.67	112.78 ± 45.87	124.65 ± 49.87	NS
Lactate dehydrogenase (U/l)	432.44 ± 278.53	361 ± 234.79	539.60 ± 306.45	< 0.001
Calcium (mg/dl)	8.01 ± 1.05	8.21 ± 0.95	7.72 ± 1.14	0.02
Inetrleukin-6	154.31 ± 77.89	101.82 ± 34.77	193.94 ± 42.52	< 0.001

Data expressed as mean (±SD). *P* value > 0.05 is not significant (NS).

Accuracy of CRP and IL-6 in prediction of mortality in patients with COVID-19 in ICU

CRP, at cutoff > 49 mg/dl, had 82.5% sensitivity and 73.3% specificity for prediction of mortality among patients with COVID-19 in ICU patients with overall accuracy of 77%. While IL-6, at cutoff > 70 pg/ml, had 88% sensitivity and 75% specificity with overall accuracy of 80% (Table 3 and Figure 1).

Table 3. Accuracy of CRP and IL-6 in prediction of mortality in patients with COVID-19 in ICU.

	C-reactive protein	Inetrleukin-6
Sensitivity	82.5%	88%
Specificity	73.3%	75%
Positive predictive value	67%	70.1%
Negative predictive value	86%	90.4%
Accuracy	77%	80%
Cutoff point	> 49	> 70
Area under the curve	0.80	0.82

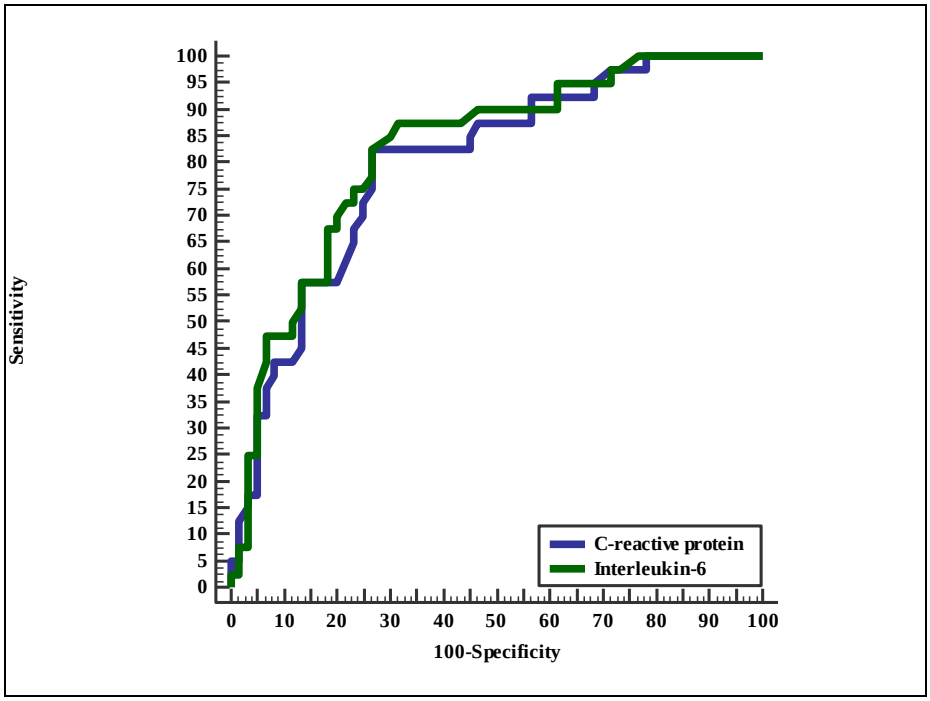

Figure 1. Accuracy of CRP and IL-6 in prediction of mortality in patients with COVID-19 in ICU.

Multivariate regression analysis for prediction of mortality in the patients

Based on data of the current study, risk factors for morality among patients with COVID-19 infection in ICU were older age (>60 years), presence of diabetes mellitus, chest diseases, and high levels of CRP, and IL-6 (Table 4).

Table 4. Multivariate regression analysis for prediction of mortality in the patients.

	Odd's ratio	95% confidence interval	*P* value
Age (> 60 year)	1.05	1.01-2.34	0.04
Diabetes mellitus	4.56	2.34-6.56	< 0.001
Chest diseases	6.41	3.45-8.45	< 0.001
CRP > 49 mg/dl	2.02	2.01-4.11	0.03
IL-6 > 70 pg/ml	3.34	2.34-5.45	< 0.001

P value was significant if < 0.05. CRP: C-reactive protein; IL-6: interleukin-6.

Discussion

The COVID-19 pandemic has been regarded as a severe public health issue that has resulted in hundreds of deaths worldwide.[6] In the present study, the mean age of non-survivors was higher and predicts mortality. In line with these findings, a study found that non-survivors were 64 (58–70) years of age, while survivors were 60 (55–65) years of age.[7] In another study there was also an increase in mortality in admitted patients as they got older, from 11% in those aged 65–69 to more than 50% in those aged 80 or beyond.[8] This may be due to the existence of several comorbidities as well as a poorer immune response to infection as a result of getting older and having less body fitness to act against viral infection.[9-11]

In this study DM was associated with mortality. Several studies provided data to support that comorbid diabetes could predict COVID-19 course and prognosis as it was found to have a detrimental impact on medical consequences, including mortality.[12-17] In general, diabetes mellitus is linked to a reduction in viral clearance, immunological dysfunction, and an increased vulnerability to inflammation and infectious illnesses.[18-20]

We found also that chest disease was associated with mortality. This is in accordance with some previous studies, they found that comorbid chronic obstructive pulmonary disease (COPD) in COVID-19 patients had a significant in-hospital death rate.[21-24] Another study showed that COPD was the most important risk factor for hospitalization, ICU stay, and mortality among COVID-19 patients.[25] COVID-19 patients with concomitant COPD and chronic respiratory disease were more likely to develop a more severe or possibly fatal COVID-19 because of their chronic inflammatory state, limited respiratory capacity, and increased vulnerability to other respiratory infections.[26, 27]

Although we found that non-survivors had significantly higher leucocytes and neutrophils with lower lymphocytes but none of them was predictors for mortality based on regression analysis. A previous study reported that individuals with a greater total leucocytic count on admission had a worse prognosis, whereas patients with low total leucocytic count had a better prognosis.[28] Other studies stated that non-survivors had considerably more leucocytes, neutrophils, and lower lymphocytes, all of which were linked to mortality.[29, 30] Raised neutrophils count in patients with COVID-19 infection with subsequent overproduction of pro-inflammatory mediators was proposed as a primary cause of severity and mortality in COVID-19.[31] Meanwhile, direct viral damage could be a cause of lymphocytes reduction.[32]

In our study, ferritin was also significantly high in non-survivors. Ferritin is a storage marker for iron. It is, nevertheless, an acute-phase reactant, whose level rises during viral and non-infectious processes of acute inflammation. In cases of COVID-19 infection, significant increases have been documented.[33] Ferritin levels were found to be higher in non-survivors and severely ill patients.[34]

About the liver function in this study the non-survivors had significantly higher aspartate transaminase (AST) and alanine transaminase (ALT). Two other investigations reported similar observations to our findings, as they found that increased AST suggested greater COVID-19 severity, although their ALT alterations were not statistically significant.[35,36]

It has been reported that on admission, COVID-19 causes increased liver function tests (e.g., AST, ALT, gamma-glutamyl transferase, total bilirubin) in more than half of the patients.[37,38] Elevation of the AST-dominant aminotransferase showed the severity of the disease and actual hepatic damage.[37, 38] An elevation in ALT was detected in COVID-19 patients with bacterial super illness as reported in an article.[39]

In our study the calcium level was significantly lower among non-survivors (Table 2). Another study reported similar observation to ours, as non-survivors had lower calcium levels.[29] Another study also showed a significant association between COVID-19 severity and decreased serum calcium.[40] Changes in intracellular calcium homeostasis can activate inflammatory pathways, resulting in an increase in inflammatory markers such as interleukin-1 (IL-1), IL-6 and tumor necrosis factor (TNF).[41,42] Another study reported that in both mild/moderate and severe/critical cases, the proinflammatory cytokine IL-6 was linked to calcium changes.[43]

In our study, LDH was significantly higher among non-survivors. LDH is a carbohydrate metabolism enzyme that converts lactate and pyruvate into glucose. It is abundant in human cells, and its level rises in a variety of disorders that damage human cells, such as cancer and SARS-CoV-2 infection.[44] LDH was identified as a predictive factor in studies, implying that LDH levels rises with cellular damage in severe COVID-19.[45-47]

In our study, non-survivors had significantly higher D-dimer. COVID-19 can damage vascular endothelium, activate coagulation factors, and deposit fibrin intravascularly as a life-threatening viral illness.[48] Proinflammatory cytokines have been shown to activate the coagulation cascade in critically ill individuals, particularly those with sepsis.[49] In some other studies, elevated D-dimer levels were associated to an increased risk of pulmonary embolism, severe illness, and mortality as well as admission to the intensive care unit.[47, 50-53]

We presented data to show that the international randomized ratio (INR) in non-survivors was higher than in survivors. In a multivariate logistical analysis, elevated INR was reported to be a major predictor of mortality. This could be caused by sepsis or disseminated intravascular coagulation (DIC) caused by COVID-19 infection or another bacterial infection.[54] In another study, the non-survivors of COVID-19 patients exhibited significantly higher INR and D-dimer values than the survival group, implying that COVID-19 patient death may be linked to DIC.[48]

In the present study we observed that IL6 and CRP levels were significantly higher in non-survivors and predicted mortality. Several studies reported that IL-6 was considerably raised in patients with severe COVID-19 and has the greatest contribution to the cytokine storm.[55-57] Furthermore, a study found that critical care patients had a type of cytokine structural disruption with elevated cytokine levels, particularly IL6.[58] The National Health Commission of China published four severe/critical risk parameters, including a gradual decrease in peripheral blood lymphocytes, a gradual increase in inflammatory markers (such as IL-6 and C-reactive protein), a gradual increase in lactic acid, and a rapid expansion of pulmonary lesions in a short period of time.[59]

CRP levels rise gradually during the onset of COVID-19 infection and was linked to disease severity and mortality.[47,60-63] Furthermore, CRP correlates with Hour-Glass findings in computed tomography[64] and respiratory failure.[65] Peiro et al., 2021, reported that CRP was significantly associated with mortality after 30 days.[44] Another study also reported that severely high serum ferritin and CRP were predictors of mortality in non-survivors.[66]

In the inflammatory cascade, IL-6 is upstream of CRP, so at the onset of COVID-19 disease, elevated IL-6 and CRP levels were found to be significantly related to a higher risk

of death.[67] SARS-CoV-2 infection may predominantly influence T lymphocytes, decreasing numbers of CD4+T and CD8+ T cells, as well as IFN-g release. This could explain the rise in inflammatory cytokines, which supports our findings. They may be essential because of the relationship between COVID-19 severity and these putative immunological indicators.[68] Functional exhaustions of cytotoxic lymphocytes could also be one of the underlying causes, and it could reflect a particular feature of the SARS-CoV-2 pathogenesis.[69]

The finding that severe inflammation may play a role in increasing mortality is consistent with the utility of steroids and anti-inflammatory medicines at the start of the pandemic.[31,57,70] More important, patients who were given dexamethasone had a higher chance of surviving.[71] Patients with IL-6 levels 30 times higher than normal had a dismal prognosis. This finding revealed that tocilizumab can be used as a COVID-19 treatment.[72] According to another study, the majority of patients who received immuno-inflammatory therapy had lower inflammatory indices and improved lymphopenia.[68]

Contrary to our findings, a study reported that IL-6 was not associated with the level of COVID-19 progression.[73] Hoverer, there was no discernible difference between the severe and mild groups. [73] On the other hand, COVID-19 patients with DM, had the same inflammation-related biomarkers as non-diabetic patients (e.g., IL-6). These findings suggest that patients with diabetes have a similar inflammatory response to those without diabetes, resulting in similar COVID-19-related death rates.[74]

In conclusion, our data indicated that IL6, CRP, DM and chest disease could predict mortality among critically ill COVID-19 patients. Therefore, these patients must be identified among ICU patients as early as possible.

Author Contributions

AOA and AFS, contributed to the study conception and design. AOA, AFS and DMA, contributed to material preparation, data collection and analysis. HGR, provided clinical support. DMA, wrote the manuscript draft. All authors read and approved the final manuscript.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) denies receipt of any financial support for the research, authorship, and/or publication of this article.

Ethical approval

The study was approved by the Ethics Committee of Assiut Faculty of Medicine (IRB No. 17200499).

Informed consent

A signed consent form was obtained from each study participant.

References

1. Abate SM, Checkol YA, Mantedafro B, et al. (2020). Prevalence and risk factors of mortality among hospitalized patients with COVID-19: a systematic review and meta-analysis. *Bull World Health Organ, 10.*
2. Hunter CA, Jones SAJNi. (2015). IL-6 as a keystone cytokine in health and disease. *Nature immunology*, *16*(5), 448-457.
3. Potempa LA, Rajab IM, Hart PC, et al. (2020). Insights into the use of C-reactive protein as a diagnostic index of disease severity in COVID-19 infections. *The Americ J Trop Mede & Hyg,* 103(2):561.
4. Siddiqi HK, Mehra MRJTjoh,. (2020). COVID-19 illness in native and immunosuppressed states: A clinical–therapeutic staging proposal. *The J Heart & Lung Transpl.*, *39*(5):405.
5. Abdullah YJ, Kadhim AS, Khallaf SA, et al. (2021). Serum levels of interleukin–6, ferritin, C-reactive protein, lactate dehydrogenase, D-dimer and count of lymphocytes and neutrophils in COVID-19 patients. Its correlation to the disease severity. *Annals Roman Soc for Cell Biol,* 2220-2228.
6. Marchetti MJAoh (2020). COVID-19-driven endothelial damage: complement, HIF-1, and ABL2 are potential pathways of damage and targets for cure. *Annals of hematol*, 99(8), 1701-1707.
7. Bardi T, Pintado V, Gomez-Rojo M, et al. (2021). Nosocomial infections associated to COVID-19 in

the intensive care unit: clinical characteristics and outcome. *Europ J Clin Microbiol & Infect Diss*, 40(3):495-502.

8. Becerra-Muñoz VM, Núñez-Gil IJ, Eid CM, et al. (2021). Clinical profile and predictors of in-hospital mortality among older patients hospitalised for COVID-19. *Age and Ageing,* 50(2*),* 326-334.
9. Covino M, De Matteis G, Santoro M, et al. (2020). Clinical characteristics and prognostic factors in COVID-19 patients aged≥ 80 years. *Geriats & gerontol Inter*, *20*(7), 704-708.
10. Iaccarino G, Grassi G, Borghi C, et al. (2020).Age and multimorbidity predict death among COVID-19 patients: results of the SARS-RAS study of the Italian Society of Hypertension. *Hypertension*, *76*(2), 366-372.
11. Bonanad C, García-Blas S, Tarazona-Santabalbina FJ, et al. (2020). Coronavirus: the geriatric emergency of 2020. Joint document of the Section on Geriatric Cardiology of the Spanish Society of Cardiology and the Spanish Society of Geriatrics and Gerontology. *Revista Española de Cardiología (English Edition),* 73(7), 569-576.
12. Gentile S, Strollo F, Ceriello AJDr, et al. (2020). COVID-19 infection in Italian people with diabetes: Lessons learned for our future (an experience to be used). *Diabetes Res & Clin Pract*, *162*, 108137.
13. Guo W, Li M, Dong YJDMRR. (2020). Diabetes is a risk factor for the progression and prognosis of COVID-19. *Diabetes/metabolism research and reviews*, 36(7), e3319.
14. John T-J, John KJCJoC. (2020).What is the role of angiotensin-converting enzyme 2 (ACE2) in COVID-19 infection in hypertensive patients with diabetes?. *Canadian J Cardiol*, 36(6):969. e3.
15. Wu J, Li W, Shi X, et al. (2020). Early antiviral treatment contributes to alleviate the severity and improve the prognosis of patients with novel coronavirus disease (COVID-19). *J Inter Med,* 288(1), 128-138.
16. Hill MA, Mantzoros C, Sowers JRJM. (2020). Commentary: COVID-19 in patients with diabetes. *Metabolism*, 107:154217.
17. Klonoff DC, Umpierrez GEJM. (2020). Letter to the Editor: COVID-19 in patients with diabetes: Risk factors that increase morbidity. *Metabolism*, *108*:154224.
18. Chee YJ, Tan SK, Yeoh EJJodi. (2020). Dissecting the interaction between COVID-19 and diabetes mellitus. *J Diab Invest,* 11(5), 1104-1114.
19. Hodgson K, Morris J, Bridson T, et al. (2015). Immunological mechanisms contributing to the double burden of diabetes and intracellular bacterial infections. *Immunol*, *144*(2), 171-185.
20. Mufier L, Gorter K, Hak EJCID. (2005). Increased risk of common infections in patient With type 1 and type 2 diabetes meuitus. *Clin infect dis*, 41(3), 281-288.
21. Almazeedi S, Al-Youha S, Jamal MH, et al. (2020).Characteristics, risk factors and outcomes among the first consecutive 1096 patients diagnosed with COVID-19 in Kuwait. *EClinicalMedicine*, 24:100448.
22. Lee JY, Kim HA, Huh K, et al. (2020). Risk factors for mortality and respiratory support in elderly patients hospitalized with COVID-19 in Korea. *J Korean Med Sc*, 35(23).
23. Wang K, Zuo P, Liu Y, et al. (2020). Clinical and Laboratory Predictors of In-hospital Mortality in Patients with Coronavirus Disease-2019: A Cohort Study in Wuhan, China. *Clin Infect Dis*, *71*(16), 2079-2088.
24. Wang L, He W, Yu X, et al. (2020).Coronavirus disease 2019 in elderly patients: characteristics and prognostic factors based on 4-week follow-up. *JInfect*, *80*(6), 639-645.
25. Gerayeli FV, Milne S, Cheung C, et al. (2021).COPD and the risk of poor outcomes in COVID-19: A systematic review and meta-analysis. *EClinicalMedicine*, *33*, 100789.
26. Gülsen A, König IR, Jappe U, et al. (2021). Effect of comorbid pulmonary disease on the severity of COVID-19: A systematic review and meta-analysis. *Respirol*, *26*(6), 552-565.
27. Grasselli G, Tonetti T, Filippini C, et al. (2021). Pathophysiology of COVID-19-associated acute respiratory distress syndrome–Authors' reply. *The Lancet Respiratory Medicine*, *9*(1), e5-e6.
28. Lagunas-Rangel FAJJomv. (2020). Neutrophil-to-lymphocyte ratio and lymphocyte-to-C-reactive protein ratio in patients with severe coronavirus disease 2019 (COVID-19): a meta-analysis. *J Med Virol*.
29. Pazoki M, Keykhaei M, Kafan S, et al. (2021). Risk indicators associated with in-hospital mortality and severity in patients with diabetes mellitus and confirmed or clinically suspected COVID-19. *J Diabe & Metabol Disorders*, *20*(1), 59-69.
30. Yang X, Yu Y, Xu J, et al. (2020). Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. *The Lancet Respiratory Medicine*, *8*(5), 475-481.

31. Mehta P, McAuley DF, Brown M, et al. (2020). COVID-19: consider cytokine storm syndromes and immunosuppression. *The lancet*, *395*(10229), 1033-1 34.
32. Channappanavar R, Zhao J, Perlman SJIr. (2014). T cell-mediated immune response to respiratory coronaviruses. *Immunol Res*, *59*(1), 118-128.
33. Kappert K, Jahić A, Tauber RJB. (2020). Assessment of serum ferritin as a biomarker in COVID-19: bystander or participant? Insights by comparison with other infectious and non-infectious diseases. *Biomarkers*, *25*(8), 616-625.
34. Kiss S, Gede N, Hegyi P, et al. (2020). Early changes in laboratory parameters are predictors of mortality and ICU admission in patients with COVID-19: a systematic review and meta-analysis. *Medical Microbiol & Immunol*, 210(1), 33-47.
35. Mo P, Xing Y, Xiao Y, et al. (2020). Clinical characteristics of refractory COVID-19 pneumonia in Wuhan, China. *Clinical infectious diseases*. 16:ciaa270.
36. Liu G, Zhang B, Zhang S, et al. (2021).LDH, CRP and ALB predict nucleic acid turn negative within 14 days in symptomatic patients with COVID-19. *Scottish Medical J*, *66*(3), 108-114.
37. Bloom PP, Meyerowitz EA, Reinus Z, et al. (2021). Liver biochemistries in hospitalized patients with COVID-19. *Hepatol*, *73*(3), 890-900.
38. Ali N, Hossain KJErog. (2020). Liver injury in severe COVID-19 infection: current insights and challenges. *Expert review of gastroenterol & hepatol*, *14*(10), 879-884.
39. Liu C, Wen Y, Wan W, et al. (2021). Clinical characteristics and antibiotics treatment in suspected bacterial infection patients with COVID-19. *Inter immunopharmacol*, *90*, 107157.
40. Lippi G, South AM, Henry BMJAocb. (2020). Electrolyte imbalances in patients with severe coronavirus disease 2019 (COVID-19). *Annals of clinical biochemistry*, *57*(3), 262-265.
41. Nieto-Torres JL, DeDiego ML, Verdiá-Báguena C, et al. (2014). Severe acute respiratory syndrome coronavirus envelope protein ion channel activity promotes virus fitness and pathogenesis. *PLoS pathogens*, *10*(5), e1004077.
42. Nieto-Torres JL, Verdiá-Báguena C, Jimenez-Guardeño JM, et al. (2015). Severe acute respiratory syndrome coronavirus E protein transports calcium ions and activates the NLRP3 inflammasome. *Virol*, *485*, 330-339.
43. Zhou X, Chen D, Wang L, et al. (2020). Low serum calcium: a new, important indicator of COVID-19 patients from mild/moderate to severe/critical. *Bioscience reports*, *40*(12).
44. Peiró ÓM, Carrasquer A, Sánchez-Gimenez R, et al. (2021). Biomarkers and short-term prognosis in COVID-19. *Biomarkers*, *26*(2), 119-126.
45. Deng X, Liu B, Li J, et al. (2020). Blood biochemical characteristics of patients with coronavirus disease 2019 (COVID-19): a systemic review and meta-analysis. *Clin Chem & Lab Med (CCLM)*, *58*(8), 1172-1181.
46. Kermali M, Khalsa RK, Pillai K, et al. (2020). The role of biomarkers in diagnosis of COVID-19–A systematic Review. *Life Sciences*, 254, 117788.
47. Ponti G, Maccaferri M, Ruini C, et al. (2020). Biomarkers associated with COVID-19 disease progression. *Critical rev in Clin Lab Scs*, *57*(6), 389-399.
48. Jin X, Duan Y, Bao T, et al. (2020). The values of coagulation function in COVID-19 patients. *PLoS One*, 15(10), e0241329.
49. Shorr AF, Thomas SJ, Alkins SA, et al. (2002). D-dimer correlates with proinflammatory cytokine levels and outcomes in critically ill patients. *Chest*, *121*(4), 1262-1268.
50. Aboughdir M, Kirwin T, Abdul Khader A, et al. (2020).Prognostic value of cardiovascular biomarkers in COVID-19: a review. *Viruses*, *12*(5), 527.
51. Huang I, Pranata R, Lim MA, et al. (2020). C-reactive protein, procalcitonin, D-dimer, and ferritin in severe coronavirus disease-2019: a meta-analysis. *Therapeutic advances in respiratory disease*, *14*, 1753466620937175.
52. Mestre-Gómez B, Lorente-Ramos R, Rogado J, et al. (2021). Incidence of pulmonary embolism in non-critically ill COVID-19 patients. Predicting factors for a challenging diagnosis. *J Thrombosis & Thrombolysis*, *51*(1), 40-46.
53. Zhang L, Yan X, Fan Q, et al. (2020). D-dimer levels on admission to predict in-hospital mortality in patients with Covid-19. *J Thrombosis & Haemostasis*, *18*(6), 1324-1329.
54. Merugu GP, Nesheiwat Z, Balla M, et al. (2021). Predictors of mortality in 217 COVID-19 patients in Northwest Ohio, United States: a retrospective study. *J Med Virol 93*(5), 2875-2882.
55. Gao Y, Li T, Han M, et al. (2020). Diagnostic utility of clinical laboratory data determinations for patients with the severe COVID-19. *J Med Virol. 92*(7), 791-796.
56. Wan S, Yi Q, Fan S, et al. (2020).Characteristics of lymphocyte subsets and cytokines in peripheral

blood of 123 hospitalized patients with 2019 novel coronavirus pneumonia (NCP). *MedRxiv*.

57. Xu Z, Shi L, Wang Y, et al. (2020). Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *The Lancet respiratory medicine*, *8*(4), 420-422.
58. Zhang Q, Wang Y, Qi C, et al. (2020). Clinical trial analysis of 2019-nCoV therapy registered in China. *J Med Virol. 92*(6), 540-545.
59. NHCo CJEAhwngcyscdcdfefceaeacp. (2020). New coronavirus pneumonia prevention and control program (7th).
60. Liu F, Li L, Xu M, et al. (2020). Prognostic value of interleukin-6, C-reactive protein, and procalcitonin in patients with COVID-19. *J Clin Virol*. 127, 104370.
61. Sahu BR, Kampa RK, Padhi A, et al. (2020). C-reactive protein: a promising biomarker for poor prognosis in COVID-19 infection. *Clinica chimica acta*, *509*, 91-94.
62. Shang W, Dong J, Ren Y, et al. (2020).The value of clinical parameters in predicting the severity of COVID-19. *J Med Virol. 92*(10), 2188-2192.
63. Wang G, Wu C, Zhang Q, et al., (2020). C-reactive protein level may predict the risk of COVID-19 aggravation. In *Open forum infectious diseases* (Vol. 7, No. 5, p. ofaa153). US: Oxford University Press.
64. Tan C, Huang Y, Shi F, et al. (2020). C-reactive protein correlates with computed tomographic findings and predicts severe COVID-19 early. *J Med Virol. 92*(7), 856-862.
65. Poggiali E, Zaino D, Immovilli P, et al. (2020). Lactate dehydrogenase and C-reactive protein as predictors of respiratory failure in CoVID-19 patients. *Clinica chimica acta*, *509*, 135-138.
66. Gayam V, Chobufo MD, Merghani MA, et al. (2021).Clinical characteristics and predictors of mortality in African-Americans with COVID-19 from an inner-city community teaching hospital in New York. *J Med Virol. 93*(2), 812-819.
67. Smilowitz NR, Kunichoff D, Garshick M, et al. (2021).C-reactive protein and clinical outcomes in patients with COVID-19. *Europ Heart J 42*(23), 2270-2279.
68. Castelnovo L, Tamburello A, Lurati A, et al. (2021).Anti-IL6 treatment of serious COVID-19 disease: a monocentric retrospective experience. *Medicine*, *100*(1).
69. Zheng M, Gao Y, Wang G, et al. (2020). Functional exhaustion of antiviral lymphocytes in COVID-19 patients. *Cell & Mol Immunol. 17*(5), 533-535.
70. Russell CD, Millar JE, Baillie JKJTL. (2020). Clinical evidence does not support corticosteroid treatment for 2019-nCoV lung injury. *The lancet*, *395*(10223), 473-475.
71. Horby P, Landrain MJNDPH. (2020). Low-cost dexamethasone reduces death by up to one third in hospitalised patients with severe respiratory complications of COVID-19. *N Engl J Med*, NEJMoa2021436.
72. Wu J, Shen J, Han Y, et al. (2021). Upregulated IL-6 indicates a poor COVID-19 prognosis: a call for tocilizumab and convalescent plasma treatment. *Frontiers in immunology*, *12*, 455.
73. Zheng H-Y, Zhang M, Yang C-X, et al. (2020). Elevated exhaustion levels and reduced functional diversity of T cells in peripheral blood may predict severe progression in COVID-19 patients. *Cell & Mol Immunol*. *17*(5), 541-543.
74. Liu Z, Li J, Huang J, et al. (2020). Association between diabetes and COVID-19: a retrospective observational study with a large sample of 1,880 cases in Leishenshan Hospital, Wuhan. *Frontiers in endocrinology*, 478.