

## ASSOCIATION OF INFLAMMATORY MARKERS WITH DISEASE SEVERITY AND OUTCOME IN COVID-19 PATIENTS

Aleksandra Aleksoska Gjuzelova<sup>1</sup>, Valentina Velkoska Nakova<sup>2</sup>,  
Zorica Nanovic<sup>1,3</sup>, Marija Metodieva<sup>1</sup>, Aleksandra Jorganovic Stojkoska<sup>1</sup>,  
Nikola Camurovski<sup>1</sup>, Vladimir Mitreski<sup>1</sup>, Slagjana Simeonova Krstevska<sup>3,4</sup>

<sup>1</sup> Institute of Lung Diseases and Tuberculosis, Skopje, RN Macedonia

<sup>2</sup> Goce Delcev University, Stip, RN Macedonia

<sup>3</sup> Faculty of Medicine, Ss. Cyril and Methodius University, Skopje, RN Macedonia

<sup>4</sup> University Clinic of Gynecology and Obstetrics, Skopje, RN Macedonia

**Corresponding author:** Aleksandra Aleksoska Gjuzelova, e-mail: sandra.aleksoska@gmail.com

### ABSTRACT

**Objectives:** The coronavirus pandemic was associated with a high mortality rate in the Republic of North Macedonia. Finding early markers of the disease's severity may predict outcomes and guide the treatment of the disease. The aim of our study was to evaluate the role of inflammatory markers in predicting the outcome of COVID-19 in hospitalized patients.

**Methods:** The study included 104 PCR-confirmed COVID-19 patients who underwent hospital treatment at the Institute of Lung Diseases and Tuberculosis in Skopje, North Macedonia, between November 2020 and May 2021. Inflammatory markers were assessed in all patients and correlated with the disease severity and outcome in terms of survival or death.

**Results:** IL-6 and LDH at admission were significantly elevated in patients with a severe or critical form of the disease and among non-survivors. In addition, IL-6 showed 87.9% of sensitivity and 61.8% of specificity for distinguishing non-survivors from survivors with a cut-off value of 21.7 pg/ml in the receiver operator curve (ROC). Procalcitonin was significantly increased in non-survivors. Parallel to the increase of disease severity, the values of CRP and LDH increased significantly during hospitalization.

**Conclusion:** The results of the study indicate that a significant association exists between the highly increased levels of CRP, LDH, IL-6 and procalcitonin and the severity of the disease and mortality in COVID-19 patients. Their measurements and follow-up during the course of the disease could be used as predictors for prognosis and outcome but also as a subject for targeted therapy.

**Keywords:** COVID-19, inflammatory markers, CRP, IL-6, LDH, procalcitonins

### INTRODUCTION

The corona virus disease-19 (COVID-19) is caused by severe acute respiratory syndrome-Coronavirus-2 (SARS-CoV-2), a novel coronavirus which belongs to the *Betacoronavirus*, and is responsible for the current pandemic.

Infection ranges from asymptomatic to severe and critical forms of the disease [1]. The differences in the clinical presentation are mainly attributed to the differences of the immune response [2]. Due to the critical role of inflammation and proinflammatory cytokines in the patho-

genesis and progression of COVID-19, many authors have investigated the role of inflammatory markers in predicting the severity of COVID-19 as well as the disease outcomes [3–5]. The conclusions of these studies that analyze the association of the inflammatory markers with the severity of COVID-19 are inconsistent [6].

The mortality rate from COVID-19 in our country shows higher numbers compared with other countries. Taking this as a starting point, we conducted a study to evaluate the role of inflammatory markers in predicting the outcome of COVID-19, and their use as a guide for more aggressive medical treatment which may bring a better outcome in severe or critical forms of the disease.

## MATERIAL AND METHODS

This is a retrospective study conducted at the Institute of Lung Diseases and Tuberculosis in Skopje, between November 2020 and May 2021. During this period, the Institute was operating as one of the state's COVID-19 centers in the Republic of North Macedonia and was used for diagnosis and in-hospital treatment of COVID-19 patients. One hundred and four patients, aged  $\geq 18$  years, who were treated for COVID-19 in the intensive and semi-intensive care unit, have been included in this study. The demographic laboratory data and course of disease and outcomes were collected from the medical records. In all patients, the coronavirus infection with SARS-CoV-2 was confirmed in respiratory specimens (nasal and/or pharyngeal swab) by reverse transcription polymerase chain reaction (RT-PCR) tests. At admission, all comorbidities of the patients were noted in their medical records, and blood samples were analyzed for a complete blood count, renal and liver function tests, C-reactive protein, Interleukin-6, procalcitonin, ferritin, lactate dehydrogenase (LDH), D-dimer and creatinine kinase (CK). For the analyzed variables, the reference values were as follows: CRP less than 6 mg/l, IL-6 less than 4.4pg/ml, ferritin less than 300ug/l, D-dimer less than 500ng/ml, procalcitonin less than 0.5ng/ml, LDH less than 248U/L, and CK from 24 U/L to 173U/L.

During patient follow-up, laboratory analyses were repeated based on indications and/or the medical condition of each patient. According

to WHO criteria [7], the severity of the patient's condition was classified as mild, moderate, severe, or critical. Patients' outcome in terms of survival was noted as "recovery" or "death".

### Statistical analysis

Data was analyzed using SPSS 20.0. Quantitative variables were represented as mean  $\pm$  SD or median. Qualitative data were presented as numbers and percentages. The chi-square test was used to determine the differences among the qualitative data. All research results were processed using a one-way analysis of variance (ANOVA). The Bonferroni post-hoc test was used for testing the significance between pairs of means. The receiver-operating characteristic (ROC) was done to determine the cut-off value of IL-6, ferritin and LDH order to predict the outcome in COVID-19 patients. The P-value of less than 0.05 was considered as statistically significant.

## RESULTS

Table 1 shows the demographic and laboratory analyses of a total number of 104 patients with COVID-19 (63.5% males and 36.5% females). From the total number of patients, 69.23% survived and 77.88% have comorbidities. According to WHO criteria for severity [7], 55 (52.88%) had a mild to moderate form, 14 (13.46%) were severely ill and 35 (33.65%) were critically ill patients.

**Table 1.** Demographic and laboratory analyses of patients with COVID19

	N=104 patients
<b>Age (mean <math>\pm</math>SD)</b>	58.56 $\pm$ 12.8 years
<b>Gender</b>	
Male (%)	66 (63,5%)
Female (%)	38 (36,5%)
<b>Disease severity</b>	
Mild to moderate (%)	55 (52.88%)
Severe (%)	14 (13.46%)
Critical (%)	35 (33.65%)
<b>Laboratory parameters (median)</b>	
CRP	74 mg/l
LDH	441 U/L
CK	103 U/L
IL-6	24 pg/ml
Procalcitonin	0.087 ng/ml
Ferritin	965.5 ug/L
Number of comorbid diseases median (min-max)	1 (0-4)

In male patients, LDH, IL-6, ferritin at admission, and levels of CRP during treatment were significantly increased in patients with a severe and critical form of the disease. LDH, IL-6, procalcitonin (PCT), and ferritin at admission and CRP during the treatment were significantly elevated among non-survivors. Non-survivors have significantly more comorbidities than survivors. (Table 2)

There was a statistically significant difference in the disease severity between mild to moderate and critical form, depending on the patient's age. In addition, there was a statistically significant difference in the level of CRP during the second checkup in patients with a critical form, compared to patients with a mild to moderate form and patients with a severe form of

the disease. Related to the level of LDH in the second and third checkup, there was a statistically significant difference between patients whose severity was mild to moderate, compared to patients with the severe form of the disease (Table 3). Statistically significant differences in other laboratory parameters between patients with mild to moderate, severe and critical disease forms were not found.

The ROC curve analysis of IL-6, ferritin, and LDH showed a good sensitivity of 87.9%, 75% and 88.2%, respectively, with a cut-off value of 21.7, 948, and 357, respectively, in predicting the outcome of COVID-19. Of all markers, IL-6 appears to have an 87.9% sensitivity and a 61.8% specificity (Figures 1-3).

**Table 2.** Association between disease severity and laboratory parameters

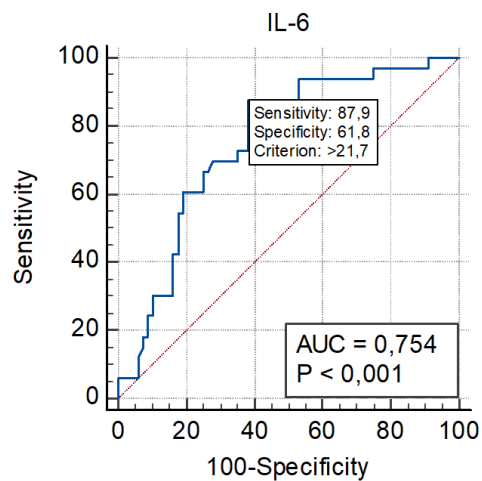
	Disease severity			P-value	Outcome		P-value
	Mild to moderate (N=55)	Severe (N=14)	Critical (N=34)		Death (N=32)	Recovery (N=72)	
Age	53.58±12.3	59.0±8.6	66.24±11.6	0.32	54.78±11.7	66.24±11.6	0.0001
Gender (male)	34 (61.8%)	9 (64.3%)	23 (67.6%)	0.01	21 (65.6%)	45 (62.5%)	0.85
CRP (mg/l)	88.49±78.9	74.20±42.6	108.58±75.9	0.27	85.29±72.1	108.58±75.9	0.135
CRP_2 (mg/l)	11.03±12.4	32.23±48.7	81.07±67.3	0.002	16.86±28.5	81.07±67.3	0.001
LDH (U/L)	381.82±141.0	534.14± 280.1	586.70± 255.4	0.046	411.30±188.8	586.70±255.4	0.001
CK (U/L)	279.09±479.2	155.33±136.6	828.80±3440	0.46	248.32±427.2	828.80 ± 3440.0	0.214
IL-6 (pg/ml)	34.12±70.9	95.40±166.3	129.68±295.9	0.07	46.26±99.62	129.68±295.9	0.038
Procalcitonin (ng/ml)	0.1096±0.093	0.1024±0.062	0.5963±1.66	0.15	0.107±0.085	0.596±1.663	0.049
Ferritin (ug/L)	963.9±661.0	965.0±820.2	1792.4±2095.3	0.045	970.0±683.9	1792.4±2095.3	0.012
Comorbidity	0.65±0.775	1.21± 1.477	1.21± 1.095	0.023	0.76±.9	1.21±1.0	0.036

The displayed results are mean±SD

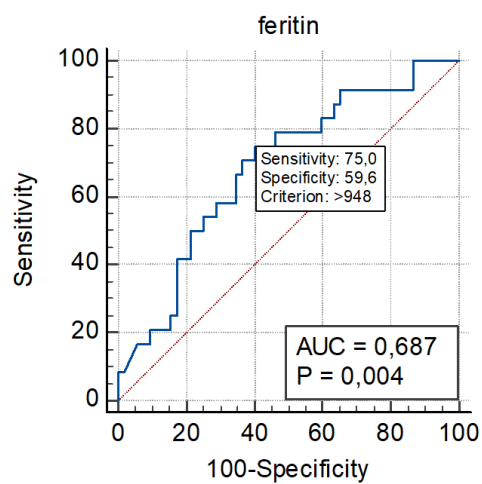
**Table 3.** Bonferroni-test for the difference in clinical presentation depending on the age and inflammatory markers

<b>Age</b>	<b>2</b>	<b>3</b>
<b>1</b>	6.043	13.285*
<b>2</b>		7.242
<b>CRP_2</b>	<b>2</b>	<b>3</b>
<b>1</b>	20.957	69.801*
<b>2</b>		48.843*
<b>LDH_1</b>	<b>2</b>	<b>3</b>
<b>1</b>	89.573	223.122*
<b>2</b>		133.549
<b>LDH_2</b>	<b>2</b>	<b>3</b>
<b>1</b>	260.013*	335.529*
<b>2</b>		75.516

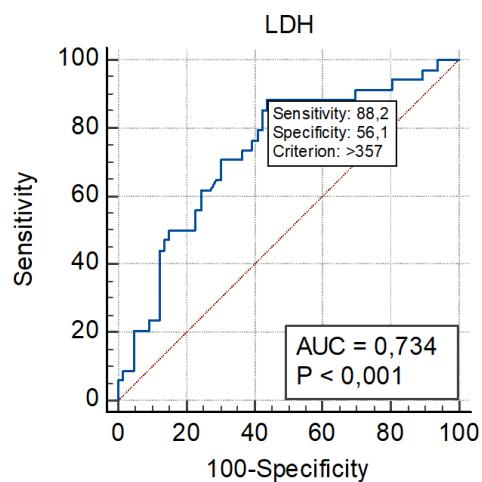
Abbreviations: 1: Mild to moderate; 2: Severe; 3: Critical condition; CRP\_2: CRP at the third checkup, LDH\_1: LDH at the second checkup, LDH\_2: LDH at the third checkup



**Figure 1.** Receiver operator curve analysis of IL-6 as a predictor of COVID-19 outcome



**Figure 2.** Receiver operator curve analysis of ferritin as a predictor of COVID-19 outcome



**Figure 3.** Receiver operator curve analysis of LDH as a predictor of COVID-19 outcome

## DISCUSSION

---

In our study, we have analyzed the association between the inflammatory markers and other laboratory parameters as well as patient outcomes, in patients with COVID-19. Our results showed elevated values of IL-6, LDH, and ferritin at admission, as well as elevated CRP during treatment in patients with a more severe clinical course and those with a poor clinical outcome (i.e. death).

Males predominate as patients with COVID-19, and are therefore associated with a more severe form of the disease, similar to the study by Sakthivadivel et al. (2021) [6]. The proportion of middle-aged patients with co-morbidities was significantly higher among non-survivors in comparison with survivors [13, 14].

Other studies showed significantly higher CRP levels in COVID-19 patients that showed severe illness compared to mild or non-severe form of the disease [9, 15-20]. In our study, we recorded a significant increase of CRP values during hospitalization. There was a statistically significant difference in the CRP values during the second follow-up after admission in patients with the critical form of COVID-19, compared to patients with a mild to moderate form and patients with a severe form of the disease. CRP at the second follow-up was significantly higher in patients with a severe form and in non-survivors, which indicates that it could be used as a risk-prediction marker of disease progression in COVID-19 patients.

LDH was not considered a classical marker of inflammation, but severe infections were found to provoke cytokine-mediated tissue damage as well as LDH release [24]. In our study, LDH levels at admission and during the entire hospitalization were significantly higher in patients with serious and critical levels of the disease. Related to the levels of LDH upon second and third follow-up, there was a statistically significant difference between patients with mild to moderate compared to patients with severe forms of the disease (Table 3). For that reason, elevated LDH levels can also be considered as a predictor of progression to severe form of the disease and higher mortality rates in COVID-19 [8, 25].

Interleukin-6 is an acute-phase inflammatory cytokine whose levels become elevated

within 2-3 hours of infection, and represents the severity of infection and poor clinical outcome in COVID-19 patients [26]. We found a significant elevation of IL-6 in severe and critical forms of the disease, and in non-survivors. Furthermore, IL-6 has the greatest sensitivity in predicting mortality among COVID-19 patients. Our results are in accordance with those of Sakthivadivel et al. (2021) [6], and demonstrate the potency of IL-6 as a predictor for disease severity and mortality in patients with COVID-19.

Many studies have also highlighted a strong association between elevated levels of procalcitonin and a severe form of COVID-19 [21-23]. Our study has found procalcitonin as a marker for the worst outcome (i.e., death): procalcitonin levels were statistically significantly increased in non-survivors.

Patients with a critical form of the disease were older and with comorbidities, with increasing levels of CRP and LDH during the disease course. This finding is in line with the recent reports showing that high levels of CRP, procalcitonin, D-dimer, LDH, and ferritin are associated with a more severe clinical course, and they are able to predict poor outcome and prognosis [8-12]. Statistically significant differences in other laboratory parameters between patients with mild to moderate, severe and critical disease forms of COVID-19 have not been found.

## CONCLUSION

---

The results of this study indicate that there is a significant association between highly increased levels of CRP, LDH, IL-6, and procalcitonin and the severity of the disease and mortality in COVID-19 patients. Their measurements and follow-up during the course of the disease could be used as predictors of prognosis and outcome, but also as a reference for targeted therapy

## REFERENCES

---

1. B1. Gao Z, Xu Y, Sun C, et al. A systematic review of asymptomatic infections with COVID-19. *J Microbiol Immunol Infect.* 2021; 54: 12-16.
2. Fajgenbaum DC, June CH. Cytokine Storm. *N Engl J Med* 2020; 383: 2255-2273.

3. Liao D, Zhou F, Luo L, et al. Haematological characteristics and risk factors in the classification and prognosis evaluation of COVID-19: a retrospective cohort study. *Lancet Haematol* 2020; 7: e671-e678.
4. Yang A-P, Liu J, Tao W, Li H, et al. The diagnostic and predictive role of NLR, d-NLR and PLR in COVID-19 patients. *Int Immunopharmacol* 2020; 84: 106504.
5. Mehta P, McAuley DF, Brown M, et al. COVID-19: consider cytokine storm syndromes and immunosuppression. *The Lancet* 2020; 395: 1033-1034.
6. Sakthivadivel V, Bohra GK, Maithilikarpagaselvi N, Khichar S, Meena M, Palanisamy N, Gaur A, Garg MK, et al. Association of Inflammatory Markers with COVID-19 Outcome among Hospitalized Patients: Experience from a Tertiary Healthcare Center in Western India. *MAEDICA – a Journal of Clinical Medicine*. 2021; 16(4): 620-627.
7. Sun H, Guo P, Zhang L, Wang F et al. Serum Interleukin-6 Concentrations and the Severity of COVID-19 Pneumonia: A Retrospective Study at a Single Center in Bengbu City, Anhui Province, China, in January and February 2020. *Med Sci Monit Int Med J Exp Clin Res* 2020; 26: e926941-1-e926941-6.
8. Henry BM, et al., Lactate dehydrogenase levels predict coronavirus disease 2019 (COVID-19) severity and mortality: A pooled analysis. *Am J Emerg Med*, 2020; 38(9): 1722–1726.
9. Huang ., et al., C-reactive protein, procalcitonin, D-dimer, and ferritin in severe coronavirus disease- 2019: a meta-analysis. *Ther Adv Respir Dis*, 2020; 14: 1753466620937175.
10. Du RH, et al., Predictors of mortality for patients with COVID-19 pneumonia caused by SARS-CoV-2: a prospective cohort study. *Eur Respir J*, 2020; 55(5).
11. Tang N, et al., Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. *J Thromb Haemost*, 2020; 18(4): 844–847.
12. Zhu Y, et al., Chinese Society of Interventional Radiology Expert Consensus on the prevention and control of COVID-19 in interventional radiology procedures (first edition). *Quant Imaging Med Surg*, 2020; 10(5): 1045–1057.
13. Qin C, Zhou L, Hu Z, et al. Dysregulation of Immune Response in Patients With Coronavirus 2019 (COVID-19) in Wuhan, China. *Clin Infect Dis* 2020; 71: 762-768.
14. Li X, Xu S, Yu M, et al. Risk factors for severity and mortality in adult COVID-19 inpatients in Wuhan. *J Allergy Clin Immunol* 2020; 146: 110.
15. Guan WJ, et al., Clinical Characteristics of Coronavirus Disease 2019 in China. *N Engl J Med*, 2020; 382(18): 1708–1720.
16. Chen N, et al., Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet* 2020:507-513.
17. Luo X, et al., Prognostic Value of C-Reactive Protein in Patients With Coronavirus 2019. *Clin Infect Dis*, 2020; 71(16): 2174–2179.
18. Chen T, et al., Clinical characteristics of 113 deceased patients with coronavirus disease 2019: retrospective study. *BMJ*, 2020; 368: m1091.
19. Gao Y, et al., Diagnostic utility of clinical laboratory data determinations for patients with the severe COVID-19. *J Med Virol*, 2020; 92(7): 791–796.
20. Hachim IY, Hachim MY, Hannawi H, Naeem KB, Salah A, Hannawi S, et al. The inflammatory biomarkers profile of hospitalized patients with COVID-19 and its association with patient's outcome: A single centered study. *PLoS ONE* 16(12): e0260537.
21. Zhang JJ, et al., Clinical characteristics of 140 patients infected with SARS-CoV-2 in Wuhan, China. *Allergy*, 2020; 75(7): 1730–1741.
22. Wang D, et al., Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus- Infected Pneumonia in Wuhan, China. *JAMA*, 2020; 323(11): 1061–1069.
23. Lippi G and Plebani M, Procalcitonin in patients with severe coronavirus disease 2019 (COVID-19): A meta-analysis. *Clin Chim Acta*, 2020; 505: 190–191.
24. Martinez-Outschoorn UE, Ketones and lactate increase cancer cell "stemness," driving recurrence, metastasis and poor clinical outcome in breast cancer: achieving personalized medicine via Metabolo-Genomics. *Cell Cycle*, 2011; 10(8): 1271–86.
25. Bartziokas K and Kostikas K, Lactate dehydrogenase, COVID-19 and mortality. *Med Clin (Engl Ed)*, 2021; 156(1): 37.
26. Bhandari S, Rankawat G, Singh A, et al. Evaluation of interleukin-6 and its association with the severity of disease in COVID-19 patients. *Indian J Med Spec* 2020; 11: 132.

## Резиме

### ПОВРЗАНОСТА НА ИНФЛАМАТОРНИТЕ МАРКЕРИ СО ТЕЖИНАТА НА БОЛЕСТА И ИСХОДОТ КАЈ ПАЦИЕНТИ СО КОВИД-19

Александра Алексоска Ѓузелова<sup>1</sup>, Валентина Велкоска Накова<sup>2</sup>,  
Зорица Нановиќ<sup>1,3</sup>, Марија Методијева<sup>1</sup>, Александра Јоргановиќ Стојкоска<sup>1</sup>,  
Никола Чамуровски<sup>1</sup>, Владимир Митрески<sup>1</sup>, Слаѓана Симеонова Крстевска<sup>3,4</sup>

<sup>1</sup> Институт за Белодробни заболувања и туберкулоза, Скопје, РС Македонија

<sup>2</sup> Универзитет Гоце Делчев, Штип, РС Македонија

<sup>3</sup> Медицински факултет, Универзитет Св.Кирил и Методиј, Скопје, РС Македонија

<sup>4</sup> Универзитетска Клиника за Гинекологија и Акушерство Скопје, РС Македонија

**Цели:** Пандемијата со корона-вирусот беше поврзана со висока стапка на смртност во РС Македонија. Со пронаоѓање маркери за рана дијагностика на тежината на болеста, може да се предвиди исходот и да се насочи третманот на болеста. Целта на студијата е да се оцени улогата на инфламаторните маркери во предвидување на исходот од КОВИД -19 кај хоспитализираните пациенти.

**Методи:** Во студијата беа вклучени вкупно 104 PCR-потврдени пациенти со КОВИД-19, кои беа хоспитално третирани во Институтот за белодробни заболувања и туберкулоза во Скопје, РС Македонија, меѓу ноември 2020 и мај 2021 год. Инфламаторните маркери беа анализирани кај сите пациенти и споредени со тежината на болеста и со исходот во однос на преживеани или починати.

**Резултати:** При прием, IL-6 и LDH беа значително покачени кај пациентите со тешка и критична форма на болеста и меѓу непреживеаните. Дополнително, IL-6 покажа 87,9 % сензитивност и 61,8 % специфичност за разликување на непреживеаните од преживеаните со гранична вредност од 21,7 според ROC-кривата. Прокалцитонин беше значително покачен кај непреживеаните. Паралелно со зголемувањето на тежината на болеста, вредностите на CRP и LDH сигнификантно се покачуваа за време на хоспитализацијата.

**Заклучок:** Резултатите од студијата покажуваат дека постои значајна поврзаност меѓу високо покачените вредности на CRP, LDH, IL-6 и procalcitonin, со тежината на болеста и морталитетот кај пациентите со Ковид-19. Нивните мерења и следење во текот на болеста може да се користат како предвидувачи на прогнозата и исходот, но и како предмет за целна терапија.

**Клучни зборови:** КОВИД-19, инфламаторни маркери, CRP, IL-6, LDH, procalcitonin