



## ASSOCIATIONS OF PROCALCITONIN, CRP AND NLR WITH MORTALITY IN HOSPITALIZED COVID-19 PATIENTS IN UNIVERSITY CLINICAL HOSPITAL MOSTAR

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**ABSTRACT** **Background:** The aim of this study was to compare the level of inflammatory parameters in 3 different groups of COVID-19 patients depending on severity of clinical manifestations and to define which inflammatory parameter can be used as predictor of clinical severity and outcome. **Methods:** A total of 51 subjects was included in this study by retrospective review of the documentation at the Clinic for Infectious Diseases of University Clinical Hospital Mostar. The following data were collected: demographic, clinical symptoms, complications, therapy, and the level of inflammatory parameters (C-reactive protein at the admission, procalcitonin, leukocytes, relative neutrophils count, relative lymphocyte count). Depending on the value of  $paO_2/fiO_2$  patients were separated into 3 groups: mild, moderate and severe group. Data entry into the tables was followed by statistical data processing using IBM SPSS Statistics v.26 and Microsoft Excel 2013. **Results:** Most frequent symptoms were fever (82% of all subjects), cough (61%), trouble breathing (55%), shortness of breath (43%), chest pain (27%), muscle pain (25%), diarrhea (20%), vomiting (6%) and headache (6%). The overall mortality of this study was 35%, with a statistically much higher mortality in severe patients. Also C-reactive protein levels were higher in severe patients. **Conclusions:** Levels of C-reactive protein on admission were significantly higher in patients who developed a severe clinical manifestations. Also, mortality in severe patients was statistically higher than the remaining 2 groups of patients. CRP is reported as a prognostic factor in the severity of clinical manifestations and mortality of hospitalized COVID-19 patients.

**KEYWORDS :** COVID-19, C-reactive protein, neutrophil to lymphocyte ratio, NLR, procalcitonin

### INTRODUCTION

The outbreak that started in the November of 2019 was caused by the novel coronavirus 2019 (COVID-19) or severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and resulted in a dangerous and deadly infection that shook public health systems, with the onset in the Wuhan, China and then expanding around the world. Highly virulent and pathogenic SARS-CoV-2 infection with a variable incubation time of 2 to 14 days, is transmitted directly by droplets or secondary contact with infected droplets. Although bats are thought to have been the primary reservoir of COVID-19, rapid interpersonal transmission is what characterizes this infection.<sup>1,2</sup>

Adults with SARS-CoV-2 infection present mostly with a triad of symptoms: fever, cough, fatigue, and only sometimes with some less common symptoms like rhinorrhea, headache, etc. Diarrhea is a rare symptom of this infection. People with a deficient immune system or chronic lung disease are more likely to develop severe viral pneumonia or bacterial superinfection that can progress to severe acute respiratory illness and death.<sup>3,5</sup> Mortality from SARS-CoV-2 infection increases exponentially with age. The mortality rate (number of deaths divided by the total number of infected) is 0.1% (1 in 1,000 infected) in children, but climbs up to 14.8% in the elderly in China, and even higher in Italy and the United States. This proportional increase in mortality with age and the fact that men die in a higher percentage than women, have attracted public attention.<sup>6-10</sup>

Procalcitonin levels have been shown to be an independent risk factor for mortality in hospitalized COVID-19 patients. Hospitalized patients with higher PCT (procalcitonin) values had a more severe clinical presentation, a more critical condition, and a higher likelihood of death.<sup>11,12</sup>

Because the level of CRP (C- reactive protein) correlates with the activity of the acute response to infection, measuring it indicates a simple, inexpensive, and rapid way to determine tissue damage at the time of measuring its level. Based on this, by measuring the level of C-reactive protein, the severity of the infection can be concluded, as well

as the damage to the internal tissues, which can be confirmed by the subsequent radiological processing.<sup>13,14</sup>

NLR (neutrophil-to-lymphocyte ratio) is an independent risk factor for the mortality of COVID-19 hospitalized patients, particularly men. The value of NLR could help in the future to identify high-risk patients with SARS-CoV-2 infection.<sup>15</sup>

A study conducted in China confirmed that baseline values of procalcitonin  $\geq 0.10$  ng / mL and CRP  $\geq 52.14$  mg / L are independent predictors of survival in patients with SARS-CoV-2 infection, however NLR does not appear to be a useful marker for predicting mortality.<sup>16</sup>

Based on all that has been published, baseline values of these parameters (PCT, CRP, and NLR) can assist the clinician in declaring severity, defining prognosis, and determining the most appropriate treatment protocol for a patient with COVID-19. That is the main contribution of this study in treating patients with SARS-CoV-2 infection.

### MATERIALS AND METHODS

#### Study Design

This was a one-center retrospective study that included 51 patients treated at two departments: COVID department as a part of the Department of Infectious Diseases and Respiratory Center as a part of the Department of Anesthesiology and Reanimatology, both in University Clinical Hospital Mostar, Mostar, Bosnia and Herzegovina. This study was approved by the Institutional Ethics Board of University Clinical Hospital Mostar, and is in full compliance with the Helsinki Declaration.

#### Participants

51 patients who were treated at the COVID department and Respiratory Center of the University Clinical Hospital Mostar during the period of the first wave of SARS-CoV-2 infection in our area, from March 15 to July 15, 2020 were enrolled in the study. The main

criterion for inclusion of the subjects is a positive PCR (polymerase chain reaction) finding from nasopharyngeal or oropharyngeal swab samples. The second inclusion criterion is hospitalization at the COVID department or Respiratory Center of the University Clinical Hospital Mostar in the period from March 15 to July 15, 2020. Only those patients who, in addition to the above two criteria, also have complete medical documentation, were included in the research.

**METHODS**

Data were collected from the medical documentation of the respondents at the Clinic for Infectious Diseases of the University Clinical Hospital Mostar. While reviewing medical records, the following data were filled in: demographic (age, gender, time from symptoms onset to hospitalization, chronic heart diseases, chronic lung diseases, diabetes, hypertension, and other comorbidities), clinical symptoms (fever, cough, anosmia, chest pain, shortness of breath, difficulty breathing, vomiting, diarrhea, muscle pain), complications (Acute Respiratory Distress Syndrome - ARDS) and level of inflammatory parameters (C-reactive protein at admission, absolute leukocyte and lymphocyte counts, relative neutrophil counts, and procalcitonin). PCT was calculated using the manufacturer's Maglumi 200 Plus automated platform, CRP was calculated using a specific protein analyzer using the manufacturer's Mindray CRP-M100 instrument, and NLR was calculated separately for each patient by dividing the absolute neutrophil count with the absolute lymphocyte count, previously detected by BC- 6900 automatic blood cell analyzer, also manufactured by Mindray.

**Statistical Analysis**

In preparation for the statistical analysis, all the above data were filled into a previously created table in MS Office Excel. Using the formulas of the Excel program, the ratio of the absolute number of neutrophils and lymphocytes was calculated, and the obtained value was entered under the variable "NLR". Patients were divided into 3 groups according to the severity of ARDS: mild, moderate, and severe. IBM SPSS Statistics v.26 was used. The results are presented in tables and graphically and are interpreted at the significance level  $P < .05$ . Continuous variables were expressed as mean (standard deviation, SD) when values were normally distributed, otherwise, the results were presented in terms of median (interquartile range, IQR). The significant differences between the groups for continuous variables were compared using one-way analysis of variance when the data were normally distributed, otherwise, the Kruskal-Wallis H-test was used. Categorical variables were described as frequency and percentages. The  $\chi^2$  (chi-square) test for independent samples and the Fisher exact test were used to process the data.

**RESULTS**

**Characteristics Among Three Groups**

The study included a total of 51 subjects in the period from March 15 to July 15, 2020 who were hospitalized at the COVID department and Respiratory Center of the University Clinical Hospital Mostar. Of the total number of patients, 27 (52.9%) were women and 24 (47.1%) were men. Of these cases, 3 patients were in the severe group, 15 patients in the moderate group, and the remaining 33 patients in the mild group. During the mentioned period, no statistical significance of the severity of the clinical picture was found among men hospitalized at the University Clinical Hospital Mostar in comparison with women, as shown in Table 1.

**Table 1. Gender and clinical differences in relation to the severity of the clinical picture**

	Severity of clinical picture (ARDSa)						$\chi^2d$	P
	Mild ARDS		Moderate ARDS		Severe ARDS			
	Nb	%c	N	%	N	%		
Gender							4,737	0,071*
Female	19	6,5	6	73,3	0	100,0		
Male	12	93,5	9	26,7	3	0,0		

<sup>a</sup> Acute Respiratory Distress Syndrome.  
<sup>b</sup> total number of patients, <sup>c</sup> percentage of patients.  
<sup>d</sup> chi square test.  
 \* indicates the value of P.

**Comorbidities Among Three Groups**

Of the total number of respondents, 38 (74.6%) had some comorbidities. High blood pressure was reported in 10 patients (20%),

diabetes in 5 patients (10%), thyroid disease in 2 patients (4%), COPD in 1 patient (2%), and Non-Hodgkin's lymphoma in 1 patient (2%). Also, 17 patients (33.3%) had 2 or more comorbidities. As shown in Table 3. there is no statistically significant difference in the presence of comorbid conditions among three groups.

**Table2. Correlation between comorbidities and severity of the clinical picture**

	Severity of clinical picture (ARDSa)						$\chi^2d$	P
	Mild ARDS		Moderate ARDS		Severe ARDS			
	Nb	%c	N	%	N	%		
Comorbidities							15,452	0,447*
W/o comorbidities	10	32,3	3	20,0	0	0,0		
Hypertension	6	19,4	4	26,7	0	0,0		
Diabetes	4	12,9	0	0,0	1	33,3		
Hypothyroidism	1	3,2	0	0,0	0	0,0		
Thyroidectomy	1	3,2	0	0,0	0	0,0		
COPD	0	0,0	1	6,7	0	0,0		
NH lymphoma	1	3,2	0	0,0	0	0,0		
2+e	8	25,8	7	46,7	2	66,7		

<sup>a</sup> Acute Respiratory Distress Syndrome.  
<sup>b</sup> total number of patients, <sup>c</sup> percentage of patients.  
<sup>d</sup> chi square test.  
<sup>e</sup> indicates patients with 2 or more comorbidities.  
 \* indicates the value of P.

**Symptoms Among Three Groups**

Statistical significance was shown for two symptoms. Subjects with moderate and severe clinical picture had shortness of breath in a significantly higher proportion ( $p < 0,009$ ), while those with mild form of the disease were significantly more likely to have muscle aches/pain ( $p < 0,045$ ). There were no statistically significant differences in other variables between groups, as shown in Table 4.

**Table 3. Differences in symptoms in relation to the severity of the clinical picture**

	Severity of clinical picture (ARDSa)						$\chi^2d$	P
	Mild ARDS		Moderate ARDS		Severe ARDS			
	Nb	%c	N	%	N	%		
Headache	2	6,5	1	6,7	0	0,0	0,631	<1*
Cough	19	61,3	9	60,0	3	100,0	1,483	0,567*
Chest pain	9	29,0	4	26,7	1	33,3	0,357	<1*
Shortness of breath	9	29,0	11	73,3	2	66,7	8,570	0,009*
Heavy breathing	14	45,2	11	73,3	3	100,0	5,151	0,066*
Diarrhea	8	25,8	2	13,3	0	0,0	1,200	0,610*
Muscle aches	12	38,7	1	6,7	0	0,0	5,887	0,045*

Data in bold indicate values of statistical significance.  
<sup>a</sup> Acute Respiratory Distress Syndrome.  
<sup>b</sup> total number of patients, <sup>c</sup> percentage of patients.  
<sup>d</sup> chi square test.  
 \* indicates the value of P.

**Levels Of Inflammatory Paramters Among Three Groups**

Statistical significance was only found for C-reactive protein ( $p < 0,023$ ). The highest levels of CRP were found in patients with severe clinical picture. Statistical significance for Procalcitonin and Neutrophil-to-Lymphocyte ratio was not found (Table 6).

**Table 4. Differences in laboratory parameters in relation to the severity of the clinical picture**

	Severity of clinical picture (ARDSa)						Hd	P
	Mild ARDS		Moderate ARDS		Severe ARDS			
	Mb	IQRc	M	IQR	M	IR		
NLRe	0,29	0,30	1,11	1,29	0,43	.	4,027	0,134*
CRPf	86,15	99,60	110,00	90,55	172,80	.	7,506	0,023*
Procalcitonin	0,06	0,08	0,20	0,50	0,70	.	5,825	0,054*

Data in bold indicate values of statistical significance.  
<sup>a</sup> Acute Respiratory Distress Syndrome.  
<sup>b</sup> median value, <sup>c</sup> interquartile range, <sup>d</sup> Kruskal-Wallis H test.

<sup>c</sup> neutrophil-to-lymphocyte ratio, <sup>f</sup> C-reactive protein.

\* indicates the value of P.

## DISCUSSION

A total of 51 subjects participated in the study conducted at the University Clinical Hospital in Mostar in the period from March 15 to July 15, 2020. The main goal was to show the correlation between 3 inflammatory parameters and the severity of the clinical picture and mortality. Statistical significance has been shown for C-reactive protein, an inflammatory protein of the acute phase, as a predictor of clinical picture severity and mortality. The value of CRP was higher in patients in the group of severe clinical picture, and lower in patients in the group of mild clinical picture. Based on this we can conclude that the value of CRP on admission correlates with the severity of the clinical picture and outcome. The statistical significance of the remaining 2 inflammatory parameters (procalcitonin and NLR) has not been demonstrated, and therefore we cannot say that their level on admission correlates with the severity of the clinical picture and outcome. The results of this study partially correlate with similar recent researches.

Liu Y. and colleagues in a study published in Wuhan showed a statistically significantly higher level of NLR in patients with a severe clinical picture, and an increased risk of death in patients who were in the group with high levels of this parameter. They also showed that the risk of death is higher in men.<sup>15</sup> Another study from Wuhan, conducted by Liu Z. and colleagues, shows several times higher risk of developing a critical severe form of the disease in patients with elevated procalcitonin levels. In conclusion, procalcitonin is cited as an independent risk factor in the death of COVID-19 patients.<sup>12</sup> A study published by Xu J. and colleagues showed statistical significance for all three inflammatory parameters: C-reactive protein, procalcitonin, and the ratio of neutrophils to lymphocytes. All three inflammatory parameters showed an upward trend from mild, over moderate, to severe clinical picture, and are described as independent predictors of clinical picture severity. Despite the confirmed correlation, no statistical significance was shown for the three listed inflammatory parameters when they were observed together. Also, in this study, NLR was not confirmed as an independent predictor of death.<sup>16</sup>

Of the total number of respondents, slightly more than half were female. The mean age was 65.7 years of age, and 78% of respondents were in the extended group of  $65.7 \pm 15.6$ . A total of 31 patients (60.7%) were in the mild group, 15 patients (29%) in the moderate group, and 3 patients (3%) in the group of severe clinical picture. 2 patients did not meet the criteria for ARDS (PaO<sub>2</sub>/fiO<sub>2</sub>), but because they were hospitalized in COVID department of University Clinical Hospital Mostar they were included in mild group. Among the symptoms, cough was the most common, followed by shortness of breath, chest pain, muscle aches, diarrhea, vomiting and headache as symptoms with the least frequency. Statistical analysis showed that subjects with moderate and severe clinical pictures were more likely to have shortness of breath, and subjects with a mild clinical picture were more likely to present with muscle pain. Three-quarters of patients had another comorbid condition with COVID-19. Despite the high incidence of other diseases, no statistically significant difference in the incidence of comorbid conditions was shown among patients in the three groups.

The research was conducted in 2 departments. The first department was an isolation ward within the Clinic for Infectious Diseases of the University Clinical Hospital Mostar, where patients with a mild and moderate clinical picture were hospitalized (65%). The second department is the Respiratory Center, as part of the Department of Anesthesia, Resuscitation and Intensive Care of the University Clinical Hospital Mostar, where patients who required invasive mechanical ventilation were hospitalized (35%).

The frequency of symptoms among subjects is fully correlated with worldwide referenced studies.<sup>12,13,15,16</sup> Xu J. and colleagues in their study also showed statistical significance for shortness of breath in patients with moderate and severe clinical picture, as well as a higher incidence of muscle pain in patients with mild clinical picture.<sup>16</sup> Liu Y. and colleagues in their study showed a higher likelihood of comorbid conditions in patients with severe clinical picture, however, in our study no significant statistical difference in the presence of comorbid conditions was demonstrated among the 3 groups of patients.<sup>15</sup> Also, mortality is most common in patients within this group.<sup>12,15,16</sup>

## CONCLUSION

C-reactive protein (CRP) shows an increasing trend from the group of patients with mild, over moderate to severe clinical picture, and on this basis correlates with the severity of the clinical picture and the fatal outcome. Procalcitonin has not been shown to be a factor correlating with the clinical picture severity and mortality. NLR has not been shown to be a factor correlating with the clinical picture severity and mortality. Shortness of breath has been shown to be a symptom that is more common in patients with a severe and moderate clinical picture. Patients with a mild form of the disease were significantly more likely to have muscle pain.

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