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The role of C-reactive protein, procalcitonin, interleukin-6 and neutrophil / lymphocyte ratio as a laboratory biomarker in COVID-19

Rusul S. F. Al-Juboori[1], and Yasmeen J. Al-Bayaa[2]

[1]Ibn Sina University of Medical & Pharmaceutical Sciences, Baghdad, Iraq.
[2]College of Medicine, University of Baghdad, Baghdad, Iraq.

Corresponding author: Yasmeen J. Al-Bayaa, College of Medicine, University of Baghdad, Baghdad, Iraq.
Email: yasmeenalbayaa@comed.uobaghdad.edu.iq

Abstract

Biomarkers such as Interleukin-6 (IL-6), Procalcitonin (PCT), C-reactive protein (CRP) and Neutrophil-Lymphocyte Ratio (NLR) have a role in the pathogenesis of severe coronavirus disease 2019 (COVID-19). The aim of this study was to explore the differences between serum levels of such biomarkers in severe and non-severe COVID-19 cases and compare them with normal people and to evaluate the sociodemographic variables and chronic diseases effect on the severity of COVID-19. The study included 160 subjects, divided into two groups, a case group of 80 patients, and a control group of 80 normal persons. The case group was divided into two subgroups: 40 severe COVID-19 patients and 40 patients with non-severe disease. Blood IL-6 was assessed by an enzyme-linked immunosorbent assay (ELISA), PCT by an immunoassay, CRP by an immunoturbidimetric assay and NLR from CBC. The levels of IL-6, PCT, CRP, and NLR were significantly higher in the case group than in control group (p= 0.001, for all). However, there was no difference between these biomarkers level in the non-severe COVID-19 subgroup and the control group (p>0.05 for all). The proportion of severe COVID-19 was significantly higher in patients aged >50 years, and in patients with chronic diseases (p=0.046 and p=0.001, respectively). We also found a strong correlation between such biomarkers and old age, and chronic diseases with the disease severity. There was a significant difference in the level of the three biomarkers (IL-6, PCT, CRP, and NLR) between patients' subgroups and the control group. In conclusion, since the levels of these biomarkers are correlated with the severity of the COVID-19 disease, and there was a difference in the levels between the groups with severe and non-severe symptoms, we suggest a role of these biomarkers in predicting the severity COVID-19 disease and its poor prognosis.

Keywords: CRP, Procalcitonin, IL-6, Neutrophil/Lymphocyte Ratio, COVID-19.

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Introduction

Three lethal pandemics have been connected to novel coronaviruses so far in the twenty-first century: severe acute respiratory syndrome (SARS), Middle East respiratory disease (MERS), and severe coronavirus disease 2019 (COVID-19). Numerous acute respiratory tract infections (ARTIs)-causing viruses are highly contagious

and/or have caused a significant number of fatalities.[1] The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been causing an outbreak of pneumonia over the world since December 2019 and was reported in Wuhan, China. The outbreak was linked to the Hunan food Market.[2] Extreme Acute Respiratory Syndrome Coronavirus 2 is a novel zoonotic coronavirus that causes the disease, which is a highly contagious viral infection. SARS-CoV-2 is cytopathic according to the laboratory researches, and this could have caused the first lung damage, as demonstrated by pathological investigations.[3] Host immune responses are stimulated as a result of viral amplification, which is thought to remove the virus and heal the patients. But it is still unclear why some people had more severe conditions. The studies hypothesized that cytokine storm plays a key role in the etiology of COVID-19 severe cases.[4]

Cytokine storms are caused by a variety of viral and non-infectious disorders, and they can harm many organs. The immune system recognizes pathogen infections through two types of responses: an innate immune response and an adaptive immune response that recognizes antigens.[5] Numerous studies have revealed that immune-inflammatory indicators in peripheral blood, including C-reactive protein (CRP), Procalcitonin (PCT), Interleukin-6 (IL-6), neutrophil count and Neutrophil-Lymphocyte Ratio (NLR) increase considerably in serious forms of COVID-19. IL-6 regulates a variety of physiologically normal and harmful biological processes in an autocrine, paracrine, and "hormone-like" way, including local and systemic inflammation, metabolism, and cancer.[6] Data from several investigations indicated elevated serum concentrations of this cytokine, especially in severe instances, support the idea that IL-6 is essential for in the immunopathogenesis of COVID-19.[7] The increase in neutrophil count indicates the severity of systemic inflammation, whereas lymphopenia indicates lymphocyte sequestration and apoptosis at the inflammation site. The combination of these two biomarkers could help predict severe COVID-19 disease.[8] The plasmatic levels of PCT which is a precursor protein of calcitonin, is generally produced by the C-cells (parafollicular) of the thyroid gland, although PCT is produced by a variety of cell types throughout the body in response to bacterial infections, CRP is a liver-produced acute-phase protein in response to IL-6 activation during infections and other inflammatory diseases. Several studies have demonstrated its utility as a predictor of severe COVID-19 types.[9]

This study was carried out to measure the levels of certain biomarkers (IL-6, PCT, CRP and NLR) in severe and non-severe COVID-19 cases and compare the collected data with a control group, also to evaluate the sociodemographic variables and chronic diseases effect on the severity of COVID-19.

Subjects and Methods

The current study was a case-control study. Blood samples were collected from 160 subjects with the age range 18-80 years. They were divided into two groups, a case, and a control group. Then the case group was categorized as severe and non-severe groups based on the World Health Organization (WHO) criteria of COVID-19 severity.

All patients were diagnosed with the reverse transcription polymerase chain reaction (RT-PCR) of nasopharyngeal swaps, the patients did the test during the incubation period of the disease between 5-14 day. The 80 patients who showed positive RT-PCR results were included as the case group, and the other 80 subjects who showed negative RT-PCR results were named the control group.

The sampling period was between January 2021 to July 2021 from several hospitals, including Al-Karama Teaching Hospital, Baghdad Teaching Hospital, Private laboratory outpatients. A blood sample of 3-5 ml was collected from each participant, about 1.8 -2 ml of whole blood was collected in EDTA tubes for hematological testing including: complete blood count (CBC) for NLR. NLR was calculated by dividing the absolute neutrophil count by the absolute lymphocyte count. The residual blood samples were kept in Gel tube for serum and plasma separation, which stored at -20°C.

Assessment of other biomarkers

The serum IL-6 biomarker was detected by using enzyme-linked immunosorbent assay (ELISA) kits (Elecsys®, Roche diagnostics, USA) on a fully automated analyzer (Roche cobas E411, USA), according to the manufacturer's instructions. The CRP was detected by an immunoturbidimetric assay (Roche C311, diagnostics USA), according to the manufacturer's instructions. Procalcitonin was detected using electrochemiluminescence immunoassay kits (Elecsys® BRAHMS Procalcitonin, Roche diagnostics, USA) according to the manufacturer's instructions.

Statistical Analyses

The Statistical Package for Social Sciences (SPSS) Version 25 was used to analyze the data. The data were presented as mean, SD, and ranges, with percentages and frequencies for categorical data. When the predicted frequency was less than 0.05, the Fisher exact test was employed instead of the Chi square test to determine whether there was a relationship between the provisional diagnosis and specific facts unbiased t-tests and analysis of variance. The continuous variables were therefore compared using (two tailed). To verify that there were differences between the research groups, post hoc tests were carried out to determine the least significant difference (LSD). A *p* value of less than 0.05 was regarded as a significant level.

Results

The age of the study sample varied from 18 to 80 years, with a mean ± SD of 49.93 ± 15.44 years. It was clear that there was a statistically significant association between severity of COVID-19 and patients' age. The proportion of severe COVID-19 was significantly higher among the patients aged > 50 years (*p*=0.046) (Table1).

Table 1. Distribution of the study patients by COVID-19 severity and age.

Variable	Severity of COVID-19		Total (%) n= 80	*p* value
	Mild to Moderate (%) n= 40	Severe (%) n= 40		
Age (Years)	no. (%)	no. (%)	no. (%)	
< 30	5 (83.3)	1 (16.7)	6 (7.5)	0.046
30 – 50	15 (62.5)	9 (37.5)	24 (30.0)	
> 50	20 (40.0)	30 (60.0)	50 (62.5)	
Total (Mean ± SD)	48.70 ± 16.85	49.01 ± 15.82	49.93 ± 15.44	

*$p \leq 0.05$ is significant.

According to the other sociodemographic and clinical characteristic, the proportion of severe COVID-19 was significantly higher among the patients stayed in hospital > 14 days (83.3%, *p*= 0.031), and those with chronic diseases (65.5%, *p*= 0.001) specially diabetes mellitus (DM) was more in the severe group. No significant association was found between the severity of disease and patients' gender (*p*= 0.072), body mass index (BMI) (p= 0.948), and smoking (*p*= 0.998) (Table 2), (Table 3).

Table 2. Comparison between the study groups by gender.

Variable	Study groups			*p* value
	Mild COVID-19 Mean ± SD	Severe COVID-19 Mean ± SD	Control Mean ± SD	
	no. (%)	no. (%)	no. (%)	
Gender				
Male	21 (52.5)	27 (67.5)	32 (40.0)	NS
Female	19 (47.5)	13 (32.5)	48 (60.0)	

p > 0.05 is not significant (NS).

Table 3. Patient distribution according to COVID-19 severity and certain sociodemographic and clinical characteristics in mild to moderate group (non-severe) and severe group.

Variable	Severity of COVID-19		Total (%) n= 80	*p* value
	Mild to Moderate (%) n= 40	Severe (%) n= 40		
BMI				
Underweight	2 (66.7)	1 (33.3)	3 (3.8)	NS
Normal	17 (50.0)	17 (50.0)	34 (42.5)	
Overweight	18 (48.6)	19 (51.4)	37 (46.3)	
Obese	3 (50.0)	3 (50.0)	6 (7.5)	
Smoking				
Smoker	14 (50.0)	14 (50.0)	28 (35.0)	NS
Non- Smoker	26 (50.0)	26 (50.0)	52 (65.0)	
Duration of Disease (Days)				
< 7	15 (62.5)	9 (37.5)	24 (30.0)	0.031
7 – 14	23 (52.3)	21 (47.7)	44 (55.0)	
> 14	2 (16.7)	10 (83.3)	12 (15.0)	
Chronic Disease				
Yes	19 (34.5)	36 (65.5)	55 (68.8)	0.001
No	21 (84.0)	4 (16.0)	25 (31.3)	

p > 0.05 is not significant (NS).

The most prevalent symptoms, in patients with severe COVID-19, were cough (100%) and fatigue (100%), followed by fever (92.5%), headache (92.5%), and shortness of breath (SOB) (90%), while 92.3% and 82.1% of cases with mild COVID-19 complained from fatigue and headache, respectively.

Laboratory findings of the inflammatory biomarkers among the study groups were as follows: in severe group, high levels of IL-6, PCT, CRP, and NLR were recorded among 35 (87.5%), 30 (75%), 35 (87.5%), and 40 (100%) of cases, respectively. In the mild group, 4 (10%), 14 (35%), 7 (17.5%), and 9 (22.5%) of cases had high levels of IL-6, PCT, CRP, and NLR, respectively. In the control group, 14 (17.5%) and 36 (45%) subjects had high levels of PCT and NLR, respectively, while all of controls had normal level of IL-6 and CRP.

Table 4. Distribution of the study groups according to inflammatory biomarkers.

Inflammatory Markers	Study Groups			Total (%) n= 160
	Mild COVID-19 n= 40	Severe COVID-19 n= 40	Controls n= 80	
IL-6				
Normal	36(90.0)	5 (12.5)	80 (100.0)	121 (75.6)
High	4 (10.0)	35 (87.5)	0 (0)	39 (24.4
PCT				
Normal	26 (65.0)	10 (25.0)	66 (82.5)	102 (63.7)
High	14 (35.0)	30 (75.0)	14 (17.5)	58 (36.3)
CRP				
Normal	33 (82.5)	5 (12.5)	80 (100.0)	118 (73.8)
High	7 (17.5)	35 (87.5)	0 (0)	42 (26.3)
NLR				
Normal	31 (77.5)	0 (0)	44 (55.0)	75 (46.9)
High	9 (22.5)	40 (100.0)	36 (45.0)	85 (53.1)

IL-6

There was a statistically significant difference in serum IL-6 level between the study groups. When compared to the controls, patients with both severe and mild forms of COVID-19 had considerably higher means of IL-6 (73.44 and 6.43 vs. 3.95, respectively, *p*=0.001). In order to corroborate the differences between the groups, post hoc tests (LSD) were performed. They revealed that the mean IL-6 was considerably greater in patients with severe COVID-19 than in patients with moderate COVID-19 (73.44 compared 6.43, *p*=0.001) and in controls (73.44 versus 3.95, *p*= 0.001). Between the mild group and controls, there was no difference in the mean of IL-6 (6.43 versus 3.95, *p*= 0.750) Table (5).

Table 5. Post hoc tests (LSD) to confirm the difference in the IL-6 mean between study groups.

	Study Groups			
	Mild COVID-19 Mean ±SD	Severe COVID-19 Mean ±SD	Control Group Mean ± SD	*p* value
IL-6	6.43 ± 5.53	73.44 ± 74.36	-	0.001
	6.43 ± 5.53	-	3.95 ± 1.27	NS
	-	73.44 ± 74.36	3.95 ± 1.27	0.001

p > 0.05 is not significant (NS).

PCT

The means of PCT levels were significantly different between the study groups. Patients in severe and mild group had a significantly higher means of PCT compared to controls (3.01 and 0.11 vs 0.06, *p*=0.001). We found that the least significant difference (LSD) of PCT mean was significantly higher in the severe group compared to the mild group (3.01 versus 0.11, *p*= 0.001), and to the control group (3.01 versus 0.06, *p*= 0.001). There was no difference in mean PCT between the mild group and controls (0.11 versus 0.06, *p*= 0.890). (Table 6).

Table 6. Post hoc tests (LSD) to confirm the difference in the PCT mean between study groups.

	Study Groups			
	Mild COVID-19 Mean ±SD	Severe COVID-19 Mean ±SD	Control Group Mean ± SD	*p* value
PCT	0.11 ± 0.13	3.01 ± 3.53	-	0.001
	0.11 ± 0.13	-	0.06 ± 0.02	NS
	-	3.01 ± 3.53	0.06 ± 0.02	0.001

p > 0.05 is not significant (NS).

CRP

The levels of CRP in both severe and mild forms of COVID-19 were significantly higher than in the control group (56.56 and 8.08 versus 2.28, *p*=0.001). The serum level of CRP was considerably higher in cases with severe COVID-19 compared to the moderate cases (56.58 versus 8.08, *p*=0.001). However, the mean CRP was not different between the mild group and controls (8.08 versus 2.28, *p*= 0.266) (Table 7).

Table 7. Post hoc tests (LSD) to confirm the difference between study groups by mean CRP.

	Study Groups			
	Mild COVID-19 Mean ±SD	Severe COVID-19 Mean ±SD	Control Group Mean ± SD	*p* value
CRP	8.08 ± 13.98	56.58 ± 48.21	-	0.001
	8.08 ± 13.98	-	2.28 ± 1.02	NS
	-	56.58 ± 48.21	2.28 ± 1.02	0.001

p > 0.05 is not significant (NS).

NLR

The NLR in this investigation revealed a statistically significant difference between the study groups. The severe group had a significantly higher means of NLR than that in mild group and controls (32.67 versus 1.98 and 2.99, respectively, *p*=0. 001). The mean NLR in the severe group was considerably greater than the mean in the moderate group. (32.67 versus 1.98, *p*= 0.001), and mean in controls (32.67 versus 2.99, *p*= 0.001). There was no difference in the mean NLR between the mild group and controls (1.98 versus 2.99, *p*= 0.684) (Table 8).

Table 8. Post hoc tests (LSD) to confirm the difference in the NLR mean between the study groups.

	Study Groups			
	Mild COVID-19 Mean ±SD	Severe COVID-19 Mean ±SD	Control Group Mean ± SD	*p* value
NLR	1.98 ± 1.99	32.67 ± 23.57	-	0.001
	1.98 ± 1.99	-	2.99 ± 1.39	NS
	-	32.67 ± 23.57	2.99 ± 1.39	0.001

p > 0.05 is not significant (NS).

Discussion

The current corona virus infection started in late 2019. On February 11, 2020, the WHO named it the epidemic COVID-19.[10] In this study 160 subjects were enrolled. Of these, 80 patients were diagnosed with COVID-19 (Case group), They were subdivided according to severity into a non-severe group which included 40 patients and 40 patients as a severe group. The other 80 individuals were included as the Control group did not have COVID-19. In this study, severe and non-severe forms of COVID-19 had a significantly higher IL-6 (*p*=0.001). Post hoc tests (LSD) showed that mean IL-6 was significantly higher in severe COVID-19 group (*p*= 0.001), but there was no difference between non-severe cases and the controls (*p*= 0.750).

Findings of a study by Liu and his colleagues in 2020 agreed with our study results. They reported that 67.9% of patients had higher levels of IL-6 at admission. The severity group had a considerably larger percentage of patients with elevated levels of IL-6 (*p*= 0.001).[11] The study by Coomes et al., 2020[12] stated that patients with significantly increased COVID-19 and IL-6 levels had poorer clinical outcomes. In patients with complex COVID-19 compared to non-complicated cases, the mean IL-6 level was 2.9 times higher (95% CI, 1.17–7.19; I 2 = 100%).[13] Several immune and non-immune cells, such as fibroblasts, mast cells, monocytes, macrophages, keratinocytes, and mesangial cells, produce and secrete the pleiotropic cytokine IL-6. The human body is primarily exposed to COVID-19 through the mouth, nose, and eyes, where it infects the host cell (alveolar type 2 cells). It attaches to its receptors (ACE2) and stimulates inflammatory responses by producing and secreting cytokines and inflammatory cytokines such as IL-6.[14]

According to PCT, the current study reported that patients with severe and non-severe group had a significantly higher means of PCT

(p=0.001). By LSD, the mean of PCT was significantly higher in severe group and in non-severe group (p= 0.001). However, the difference in means PCT between non-severe and controls was not significant (p=0.890). This result agreed to that published in the study by Sayah et al., 2021,[2] in which serum PCT was significantly higher in patients with severe COVID-19 (p<0.0001).[15]

Also, the study by Liu et al., 2020[8] found that patients with increased PCT levels was significantly higher in the severe group than in the mild group (p<0.05). Also, the results of the study by Lui et al., 2020, showed that the mean serum PCT was more than four times higher in severe patients than in moderates and over eight times higher in critical patients than in moderate patients.[11] This increase could reflect bacterial co-infection, which likely occurs later in the course of illness, or severe COVID-19.[16] The present work revealed that patients with severe and mild (non-severe) COVID-19 had a significantly higher means of CRP (p=0.001). According to LSD, CRP was significantly higher in the severe group than in the non-severe cases and in controls (p= 0.001). No difference was found between the mild group and controls (p= 0.266).

Similarly, the study by Ahnach et al., 2020, found that CRP level was significantly higher in those with severe COVID-19 (p=0.000). It was also an independent discriminator of severe illness on admission in comparison with other biological factors,[17] indicating that this biomarker can be tracked to assess disease development.[18] A highly sensitive indicator for infection, inflammation, and tissue damage, is the acute inflammatory protein NLR. In this study, the severe group had a significantly higher mean of NLR than in the mild and control groups (p=0.001). LSD showed that the mean NLR was significantly higher in the severe group than in the mild and control groups (p=0.001).

There was no significant difference between the mild group and controls (p=0.684). Findings of the study by Vafadar et al., 2021, agreed was our observation that the increased NLR, and WBC count were associated with more severe COVID-19 and a high risk of one-month mortality (p<0.05).[19] The study by Qin et al., 2020, revealed that severe cases tend to have lower lymphocytes counts, higher leukocytes count and in turn lead to a significant higher NLR (p<0.05).[18] Infectious diseases like sepsis and bacteremia, an elevated NLR shows an imbalance of the inflammatory response and could be regarded as a likely sign of disease severity.[20]

In this study, the age ranged from 18 to 80 years with mean ±SD of age of 46.96 ± 17.07 years, and the highest proportion in the case group aged >50 years. Especially severe COVID-19 was significantly higher among those aged >50 years. Moreover, males were predominant in the case group (52.5% in mild, and 67.5% in severe group). By comparison of age and gender between case and control groups, there was no difference between them (p= 0.064 and p= 0.72, respectively). In the study by Yang et al., 2020, the mean ±SD of age was 59·7 ±13·3 years, in which 52% of them were older than 60 years.[21] A male predominance was reported as they constituted 67% of patients.[21] It was reported that elderly patients are more amenable to severe COVID-19 disease than patients younger than 50 years; this may be the result of health issues and comorbidities in that population group. Moreover, male predominance reported in many studies may attributed to higher chance of infection that linked to the occupational risk factors for men in markets, being socially active and work in crowded areas.[22]

In this study, patients who stayed in the hospital longer than 14 days were significantly more likely to have severe COVID-19, and those of chronic diseases (p< 0.05), especially DM was observed in 70% of patients in the severe group. No significant association was found between severe COVID-19 with gender (p= 0.443), BMI, and smoking (p>0.05). Differently, there was no significant differences in comorbidities including hypertension, coronary artery diseases, chronic obstructive lung disease (p>0.05). However, DM was more frequent in the severe COVID-19 group (p= 0.044)[23] In the same accordance, the study by Barman et al., 2021[24] observed that severe COVID-19 disease was significantly higher in older patients in comparison to other groups of patients (p=0.001). However, there

was no significant difference in gender between study groups (p>0.05).

In the present study, all patients with severe COVID-19 had cough and fatigue (100%), fever (92.5%), headache (92.5%), and SOB (90%). The most frequent duration was 7-14 days, in 57.5% of the mild group and 52.5% of the severe group. By comparison, the most common symptoms in the study by Liu et al., 2020, were fever (71.6%), dry cough (56.8%) and dyspnea (50.5%); and the less common symptoms were fatigue (33.7%), nausea (14.7%), myalgia (14.7%).[11] In the study by Yang et al., 2020, the common symptoms were fever (98%), cough (77%), and dyspnea (63·5%).[21]

In conclusion, raised levels of IL-6, PCT, CRP, and NLR were substantially greater in COVID-19 patients (case group) compared to the control group and reflect the host's immunological responses to the coronavirus infection. There was a significant difference in the level of IL-6, PCT, CRP, and NLR between the group with severe symptoms and the group with non-severe symptoms. Such correlation between their high levels and the severity of the disease may indicate that they can play an important role as predictive biomarkers of disease severity and poor prognosis. The extent of severe COVID-19 was significantly higher among the patients aged >50 years, patients stayed in hospital > 14 days, and those who had chronic diseases specially DM. But no significant association was found between the severity of disease and patients' gender, BMI, and smoking.

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Author Contributions

YJA and RSFA; Conceptualization of the study. RSFA; methodology. YJA; validation of the study, formal data analysis. RSFA; Investigation. YJA; resources, data curation. RSFA and YJA, writing and original draft preparation. YJA; visualization, supervision. RSFA and YJA, project administration and funding acquisition.

Declaration of Conflicting Interests

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Ethical approval

The study was approved by the College of Medicine/ University of Baghdad.

Informed consent

Informed consent was obtained from study participants before being included in the study.

ORCID iD

Yasmeen J. Al-Bayaa **iD** https://orcid.org/0000-0001-7665-739X.

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