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Studying the role of Interleukin-6 (IL-6) and C-reactive protein (CRP) in patients infected with COVID-19 until recovery

Mohey H. Shikhoun1, Yasin Abdelkareem 2

1-Analysis and Laboratories Department, Higher Technological Institute of Applied Health Sciences in Sohag, Ministry of Higher Education, Cairo, Egypt.

2- Chest Diseases Department, Medicine –Al-Azhar University

*Correspondence: moheyshikhoun@gmail.com Tel.: (+01119025409)

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Abstract

Background: Researchers studying medicine are becoming concerned about the pathogen's deciding element as the coronavirus illness 2019 (COVID-19) expands globally. We postulated a relationship between the degree of viral recovery and the amount of interleukin 6 and C-reactive protein.

Aim: Study the level of interleukin-6 in the case of infection with COVID-19 with C-reactive protein and compare its level in the case of response and non-response to treatment. **Results:** The current study included 1,100 people who had signs and symptoms of a common cold and were tested for the presence of SARS-2 RNA using reverse transcriptase-polymerase chain reaction (RT-PCR). 660 cases came back positive, and all cases underwent laboratory tests. Out of 660 patients, 50 were selected. Patients with COVID-19 infection and 50 people without COVID-19 infection were randomly assigned to study interleukin and C-RP levels in these patients. Our results showed that there are high statistical significances **(P value < 0.05)** in the level of IL-6 and the level of C-RP between patients infected with COVID-19 before and after treatment, and there are also high statistical significances **(P value < 0.05)** between patients who responded and those who did not respond to treatment.

Conclusion: Il-6 and C-RP levels increase during COVID-19 infection and gradually decrease with treatment until complete recovery. In particular, the levels of Il-6 and C-RP increased significantly in patients who did not respond to treatment, while they decreased in patients who responded to treatment.

Keywords: COVID-19; SARS-CoV-2; Interleukin-6 (IL-6); diagnosis; laboratory findings.

INTRODUCTION:

A class of single-strain RNA viruses known as coronaviruses (COVID-19) can infect humans and other hosts with respiratory diseases **[1]**. A novel beta coronavirus known as SARS-CoV-2 first surfaced in China before the end of 2019 and has since spread to about 90 million people worldwide, killing over 1.9 million and igniting a pandemic known as COVID-19 (coronavirus disease 2019) **[2]**. While 20% of patients experience severe pathology, such as acute bilateral pneumonia, which can result in acute respiratory distress syndrome and multi-organ failure, the majority of cases have moderate symptoms. The chance of developing a serious illness and passing away rises with age and the presence of comorbidities **[3]**.

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On February 14, 2020, the first case of a foreigner in Egypt was found. It took over two weeks to find the Egyptian cases then different COVID-19 variants were diagnosed in Egypt with COVID-19 JN.1 variant infection on 28 December 2023 [**4**]. Research indicates that other coronaviruses exhibit a widespread inflammatory storm **[5]**. Additionally, COVID-19 was associated with higher levels of inflammatory markers such as C-reactive protein (CRP) and interleukin-6 (IL-6) **[6]**. The reduction of albumin levels in inflammatory conditions was confirmed in individuals with severe COVID-19 **[7]**.

The prototypical cytokine IL-6 is essential for host defense because of its pleiotropic activity and functional redundancy **[8].** A wide range of cells, including immune-mediated cells, mesenchymal cells, endothelial cells, fibroblasts, cancer cells, and even many other cells, produce IL-6 quickly in response to infection and tissue damage. This protein enhances host defense by inducing acute phase reactions, hematopoiesis, and immune responses **[9]**.

Immediate-phase proteins, including serum amyloid A (SAA) and C-reactive protein (CRP), are produced in enormous quantities when IL-6 enters the liver through the bloodstream during an immediate inflammatory response. In addition, aberrant IL-6 production contributes pathologically to autoimmunity and chronic inflammation. If the high SAA concentration continues, it will encourage the development of problems from chronic inflammatory diseases as well as organ failure **[10]**.

It has been demonstrated that infections with SARS and MERS cause both innate and adaptive immunological reactions **[11, 12]**. In other words, changes in peripheral blood cells may be an indication of immunological damage brought on by a viral infection. During viral infection, lymphocytes specifically, SARS-CoV-2 lymphocytes are essential for preserving immunological homeostasis **[13]**. Numerous cohort studies have demonstrated that lymphopenia is a reliable indicator of prognosis for COVID-19 patients **[14, 15]**.

Researchers studying pathology have found that while the adaptive immune system is not overrepresented in pulmonary fibrosis, the innate immune system is. SARS-CoV-2 is equally challenging to identify. In actuality, one would anticipate that the adaptive immune system would react after the innate immune system. It seems that COVID-19 functions in the opposite way from normal. The innate immune system affects the lungs after a significant adaptive immune system is first identified. In the context of the SARS-CoV-2-caused disease COVID-19, CRP plasma levels rise to levels similar to those of bacterial illnesses **[16, 17]**.

Moreover, CRP levels have an odds ratio of 18.9 **[18]** and have been demonstrated to be a reliable indicator for a number of harmful processes, including the need for mechanical ventilation **[17, 19]**. Consequently, early in the pandemic, therapeutic targeting of CRP was suggested **[20, 21]**. An extracorporeal therapy called CRP apheresis lowers CRP plasma levels in a focused, non-invasive way. Consequently, CRP can now be precisely and therapeutically targeted **[22, 23]**. It was just recently authorized as a treatment for severe pneumonia brought on by SARS-CoV-2 **[20, 24]**.

Among hematological markers, lymphopenia is unquestionably connected with the severity of the disease; patients who died from COVID-19 had much lower lymphocyte counts than survivors. Indeed, lymphocyte repletion might be an important part of the healing process **[25]**. Although their significance is still understood, other blood components, including platelets,

neutrophils, eosinophils, white blood cells, and CD8 cell counts, were partially predictive of mild versus severe COVID-19. It has been noted that ICU patients have greater levels of granulocyte colony-stimulating factor (G-CSF), which is correlated with the severity of their disease **[**26**]**.

Material and methods

Collection and processing of blood samples

The study took place from November 2020 to July 2021, and every sample came from Egypt's Sohag Fever Hospital. 1100 patients with common cold symptoms were chosen for our study, and each patient had an interview as well as fill out a questionnaire with their age and sex. Each subject's cubital vein was venipuncture to obtain blood samples. After soaking 1% iodine and 70% isopropyl alcohol in water for one minute, the site was carefully cleansed before being allowed to dry. A sterile syringe and needle were used to extract around 5 milliliters of blood, which was then poured into clean plastic to prevent contamination of the area. The serum produced by centrifuging the blood samples for ten minutes at 4000 rpm was kept at -70°C.

Collecting Respiratory Specimens for Real-Time PCR Analysis of SARS-2 RNA

As part of infection control procedures, the hospital care physician collected nasal swabs. A sterile swab was used to take the sample from the nasopharynx. The nasopharyngeal swab's flexible plastic arm is used to put the swab into the nostril parallel to the mouth and down to a depth equal to the openings of the external and nasal airways. After a brief rotation to allow the swab to absorb nasal secretions, it is gradually removed.

Phosphate buffer containing 1 g/L proteinase K should be used to liquefy the sputum sample. The lid should then be closed and covered with a sealing film. After obtaining pharyngeal swabs, the tail was thrown away, the cap was tightened, and the sample was transferred into a disposable viral sampling tube filled with saline.

Real-time RNA SARS-2 by PCR, a model of real-time DNA technology, SARS-Cov2 fluorescent PCR Kit (Russian manufactured, lot 0921741), Maccura Biotechnology, Co., Ltd.

Testing for total leucocytic count (TLC), lymphocyte cells (TLC), platelets (PLTs), hemoglobin (Hb%), red blood cells (RBCs), and platelets

Automation model for DIRUI Hematology Analyzer: BCC-3000B.

Lot reagents: DIRUI 20210226.

When a blood sample is mixed and placed on a rack in an automated analyzer, analysis can start. The device analyzes several components in the blood using flow cells, photometers, and apertures. The component that counts cells counts the various types and quantities of cells that make up blood. The amount of hemoglobin is measured by a specialized photometer known as a hemoglobin meter. Use a diluent to lyse the red blood cells, then pump the mixture into a spectro-photometric measuring cuvette to do this. The color shift of the lysate indicates the concentration of hemoglobin in the blood.

By aspirating a little sample, diluting it, and then running it through an aperture and a laser flow cell, blood cells are counted using flow cytometry. The number of cells that pass through the opening is measured and recorded by sensors. The two most commonly utilized types of sensors are electrical impedance and laser light detectors. The technology determines the type of blood cell by looking at details about the size and properties of light as it travels through the cells. Different red blood cell index characteristics obtained from supplementary CBC values are often presented in addition to cell counts and hemoglobin.

Test of Inflammatory Index for C-reactive protein (CRP)

The blood sample in the test vial is combined with the detection buffer in the sandwich immuno-detection method used by the Ichroma TM CRP Kit

(Lot reagents: CRQBK06). As a result, the fluorescently tagged anti-CRP antibody in the buffer attaches itself to the CRP antigen in the blood sample. The sample mixture is placed on the immobilized anti-CRP sandwich pair antibody, which allows it to travel across the matrix of the test cartridge and capture the complexes between the detection antibody and CRP. The fluorescence intensity is converted into a CRP concentration using a pre-programmed calibration method. The test findings are shown to the reader as ng/mL for CRP.

Procedure: In a tube containing the detection buffer, 30 µl of serum is added and mixed about ten times. Next, 75 µl of a sample combination is added, loaded into the sample well on the test cartridge, and incubated for three minutes at room temperature. The I Chroma apparatus, model I Chroma PCT, was used to prepare the reaction.

Interleukin-6 (IL-6) Quantitative Test

Fluorescence immunoassay technology is the foundation of the FinecareTM IL-6 Rapid Quantitative Test. The sandwich immunodetection approach is used in the FinecareTM IL-6 Rapid Quantitative Test. The fluorescence-labeled detector IL-6 antibodies on the sample pad attach to the analyte in the sample when it is put on the test cartridge's sample well, forming immune complexes. The complexes of detector antibodies and IL-6 antigens are captured by IL-6 antibodies that have been immobilized on the test strip as they travel over the nitrocellulose matrix through capillary action. Therefore, the more complexes aggregated on the test strip, the more IL-6 antigens there were in the blood collection. The quantity of IL-6 that is collected is shown by the detector antibodies' fluorescence signal intensity.

Procedure: 15 minutes of room-temperature incubation were required after adding 75 µL of serum to the detection buffer tube and mixing it roughly ten times. Next, 75 µL of a sample combination was added and loaded into the sample well on the test cartridge. The Fine CareTM FIA System (Guangzhou Wondfo Biotech Co., Ltd.) instrument (LOT: F25114604AD) was used to prepare the reaction.

Statistical Analysis:

A statistical program called SPSS version 21 was used to enter and code the data. The data were described using the mean and standard deviation for quantitative variables and the relative frequencies (percentages) and frequencies (number of cases) for categorical variables. For two-group comparisons, the nonparametric Mann-Whitney U test was employed.; the nonparametric Kruskal-Wallis test was used for comparisons involving more than two groups. Depending on the circumstances, either the chi-square or Fisher's exact test were employed. We calculated the 95% confidence intervals for the odds ratios. P-values were considered statistically significant if they were less than 0.05.

Results:

To detect COVID-19, which produces symptoms like fever, coughing, and dyspnea, swabs were taken from 1100 people. RT-PCR SARS2 RNA supported our results, which showed that 660 (60%) of the patients were positive and 440 (40%) were negative.

Table 1: COVID-19 RNA molecular detection

No. patients / Total	Percent (%)	SARS2-RNA by PCR
440 / 1100	40 %	Negative
660 /1100	60 %	Positive

Out of 660 patients who received nasal swabs and were moved to the intensive care unit to get therapy, 50 patients were randomly chosen to be infected with COVID-19. The treatment was overseen by the Egyptian Ministry of Health. Patients underwent immunological and chemical testing both before and after treatment, and RNA SASRS2 PCR was used to confirm the patient's reaction to the medication. 50 individuals were chosen who were not infected with COVID-19; PCR was used to confirm this, and they also underwent chemical and immunological testing.

Our immunological investigation of the interleukin-6 test revealed patients who had both COVID-19 infection and had not received treatment, as the results demonstrated a significant difference between the pre-and post-treatment periods (P value < 0.05), with the level of interleukin-6 increasing during COVID-19 infection and progressively decreasing with treatment until full recovery, as well as the CRP test. According to Table 4 and Figure 1 of our study, there is a significant difference between COVID-19 before and after therapy (P value < 0.05). Our study of blood cells' hematological properties showed a significant difference in lymphocyte levels between patients who were infected with COVID-19 before and after treatment exposure (P value < 0.05). The level of lymphocytes decreases during COVID-19 infection (before treatment) and progressively increases with treatment until full recovery (after treatment). Unlike the hemoglobin level percentage, our investigation demonstrated statistically significant variations between patients before and after treatment exposure, during COVID-19 infection, and after treatment. Whereas there are no significant differences in total white blood cells (WBCS), monocytes, neutrophils, red blood cells (RBCS), or blood platelets (PLTS) (P value > 0.05) **(Table 2) (Figure 1)**.

Our immunological study on the interleukin-6 test demonstrated patients who were COVID-19 infected and before exposure to treatment, as the research showed that there is a significant difference in cases of infection (before treatment) and among patients who are not infected with COVID-19 (Normal patients) (P value < 0.05). Where the level of interleukin-6 increases during infection with COVID-19, as well as the CRP test. Our study showed that there is a significant difference (P value < 0.05) **(Table 3 and Figure 2)**. Our study on blood cells showed that in patients who were COVID-19 infected and before exposure to treatment, there was a significant difference in Lymphocyte cells (P value < 0.05) in cases of infection (before treatment) and among patients who were not infected with COVID-19 (normal patients), where the level of Lymphocyte cells decreased during COVID-19 infection. While in hemoglobin (Hb%), total white blood cells (WBCS), neutrophils cells, monocyte cells, red blood cells (RBCS), and blood platelets (PLTS), there are no significant differences (P value > 0.05) **(Table 3 and Figure 2).**

Table 2: Interleukin-6, C. Reactive Protein and Hematological parameters analysis of studied Patients before and after treatment.

		Before Treatment	After treatment	T. Value	P. Value	Sig.
IL-6 (Pg/ml)	Mean ±SD	84.02 ± 25.02	13.88 ± 7.21	20.243	0.000	**H. S**
Hb (g/dl)	Mean ±SD	12.25 ± 2.02	9.87 ± 1.072	5.305	0.000	**H. S**
RBCS (10^6/UL)	Mean ±SD	5.07 ± 0.54	5.06 ± 0.58	0.877	0.162	**N. S**
PLTS (10^3/UL)	Mean ±SD	280.6 ± 94.75	285.57 ± 95.27	-1.029	0.343	**N. S**
WBCS (10^3/UL)	Mean ±SD	7271.4 ± 1812.7	7100.0 ± 1961.3	1.137	0.299	**N.S**
Lymph. %	Mean ±SD	20.2 ± 6.6	32.7 ± 3.71	-14.67	0.000	**H.S**
Neutrophils %	Mean ±SD	52.8 ± 14.42	52.3 ± 13.54	0.706	0.484	**N.S**
Monocytes %	Mean ±SD	8.98 ± 5.95	9.63 ± 5.45	0.497	0.622	**N.S**
C-RP mg/L	Mean ±SD	56.31 ± 39.53	6.95 ± 3.79	9.551	0.000	**H.S**
N.S: Non-Significant **S:** Significant **H.S:** Highly Significant						

Table 3: Interleukin-6, C-reactive protein, and hematological parameters analysis of studied patients in cases of infection (before treatment) and among patients who are not infected with Coronavirus (normal patients).

		Before Treatment	Normal Patients	T. Value	P. Value	Sig.
IL-6 (Pg/ml)	Mean ±SD	84.02 ± 25.02	11.35 ± 5.33	22.325	0.000	**H.S**
Hb (g/dl)	Mean ±SD	12.25 ± 2.02	12.52 ± 2.063	-0.503	0.620	**N.S**
RBCS (10^6/UL)	Mean ±SD	5.07 ± 0.54	4.90 ± 0.473	0.658	0.535	**N.S**
PLTS (10^3/UL)	Mean ±SD	280.6 ± 94.75	279.20 ± 94.16	0.032	0.976	**N.S**
WBCS (10^3/UL)	Mean ±SD	7271.4 ± 1812.7	7757.14 ± 1560.83	-0.524	0.619	**N.S**
Lymph. %	Mean ±SD	20.2 ± 6.6	31.866 ± 5.88	-9.723	0.000	**H.S**
Neutrophils %	Mean ±SD	53.8 ± 14.42	51.66 ± 12.14	0.900	0.373	**N.S**
Monocytes %	Mean ±SD	8.98 ± 5.95	10.11 ± 5.09	-0.907	0.369.	**N.S**
C-RP mg/L	Mean ±SD	56.31 ± 39.53	5.02 ± 2.33	9.090	0.000	**H.S**
N.S: Non-Significant **S:** Significant **H.S:** Highly Significant						

Our immunological study on the interleukin-6 test showed patients who were infected with COVID-19 and after exposure to treatment, as the study showed that there is a significant difference (P value < 0.05) in cases of infection (after treatment) and among patients who are not infected with COVID-19 (normal patients). where there is a slight increase in the level of interleukin-6 during infection with COVID-19 after treatment, as well as the CRP test. **(Table 4 and Figure 3)**

Our hematological parameters study on blood cells showed that in patients who were infected with COVID-19 and after exposure to treatment, there is a significant difference in hemoglobin levels (P value < 0.05) in cases of infection (after treatment) and among patients who are not infected with COVID-19 (normal patients), where the level of hemoglobin decreases during infection with COVID-19 and treatment. while in red blood cells (RBCS) and total white blood cells (WBCS), neutrophil cells, lymphocyte cells, monocyte cells, and blood platelets (PLTS) there are no significant (P value > 0.05) **(Table 4 and Figure 3).**

Our investigation into the interleukin-6 levels in COVID-19-infected patients exposed to treatment demonstrated a significant statistical difference between patients who responded to treatment and those who did not (P value < 0.05). Specifically, interleukin-6 levels significantly increased in non-responding patients while decreasing in treatment-responding patients, and the same was true for CRP levels. Additionally, our study demonstrated the level of lymphocyte cells in COVID-19-infected patients who were treated and it also demonstrated a significant statistical difference between patients who responded to treatment and those who did not (P value < 0.05); in the former group, lymphocyte cell levels significantly decreased, whereas in the latter group, levels increased, While there are no significant differences (P value > 0.05) in hemoglobin (Hb%), red blood cells (RBCS), total white blood cells (WBCS), neutrophil cells, monocyte cells, and blood platelets (PLTS), **(Table 5 and Figure 4).**

Table 4: Interleukin-6, C. reactive protein, and hematological parameters were analyzed in patients who were either infected with COVID-19 (after therapy) or who were not (normal patients).

		After Treatment	Normal Patients	T. Value	P. Value	Sig.
IL-6 (Pg/ml)	Mean ±SD	13.88 ± 7.21	11.35 ± 5.33	2.100	0.041	**S**
Hb (g/dl)	Mean ±SD	9.87 ± 1.072	12.52 ± 2.063	-5.74	0.000	**H.S**
RBCS (10^6/UL)	Mean ±SD	5.06 ± 0.58	4.90 ± 0.473	0.535	0.612	**N.S**
PLTS (10^3/UL)	Mean ±SD	285.57 ± 95.27	279.20 ± 94.16	0.146	0.889	**N.S**
WBCS (10^3/UL)	Mean ±SD	7100.0 ± 1961.3	7757.14 ± 1560.83	-0.690	0.516	**N.S**
Lymph. %	Mean ±SD	32.7 ± 3.71	31.866 ± 5.88	0.87	0.389	**N.S**
Neutrophils %	Mean ±SD	52.3 ± 13.54	51.66 ± 12.14	0.264	0.973	**N.S**
Monocytes %	Mean ±SD	9.63 ± 5.45	10.11 ± 5.09	-2.251	0.029	**N.S**
C-RP mg/L	Mean ±SD	6.95 ± 3.79	5.02 ± 2.33	2.905	0.005	**S**

N.S: Non-Significant **S:** Significant **H.S:** Highly Significant

Table 5: Comparing interleukin-6, C-reactive protein, and hematological parameters for patients with COVID-19 who responded to treatment and patients who did not.

Biochemical test in responded and non-responded patients		**After Treatment for responded patients**	**After Treatment for non-responded patients**	**T. Value**	**P. Value**	**Sig.**
IL-6 (Pg/ml)	Mean ±SD	13.88 ± 7.21	69.243 ± 19.32	-8.205	0.000	**H.S**
Hb (g/dl)	Mean ±SD	9.87 ± 1.072	10.014 ± 0.710	-0.637	0.548	**N.S**
RBCS (10^6/UL)	Mean ±SD	5.06 ± 0.58	4.93 ± 0.68	0.923	0.391	**N.S**
PLTS (10^3/UL)	Mean ±SD	285.57 ± 95.27	278.16 ± 104.85	0.438	0.677	**N.S**
WBCS (10^3/UL)	Mean ±SD	7100.0 ± 1961.3	7400 ± 7271.43	-1.137	0.299	**N.S**
Lymph. %	Mean ±SD	32.7 ± 3.71	19.94 ± 9.50	4.795	0.003	**S**
Neutrophils %	Mean ±SD	52.3 ± 13.54	51.86 ± 15.55	1.055	0.332	**N.S**
Monocytes %	Mean ±SD	9.63 ± 5.45	10.043 ± 6.47	-0.136	0.896	**N.S**
C-RP mg/L	Mean ±SD	6.95 ± 3.79	65.87 ± 22.87	-6.85	0.000	**H.S**
N.S: Non-Significant **S:** Significant **H.S:** Highly Significant						

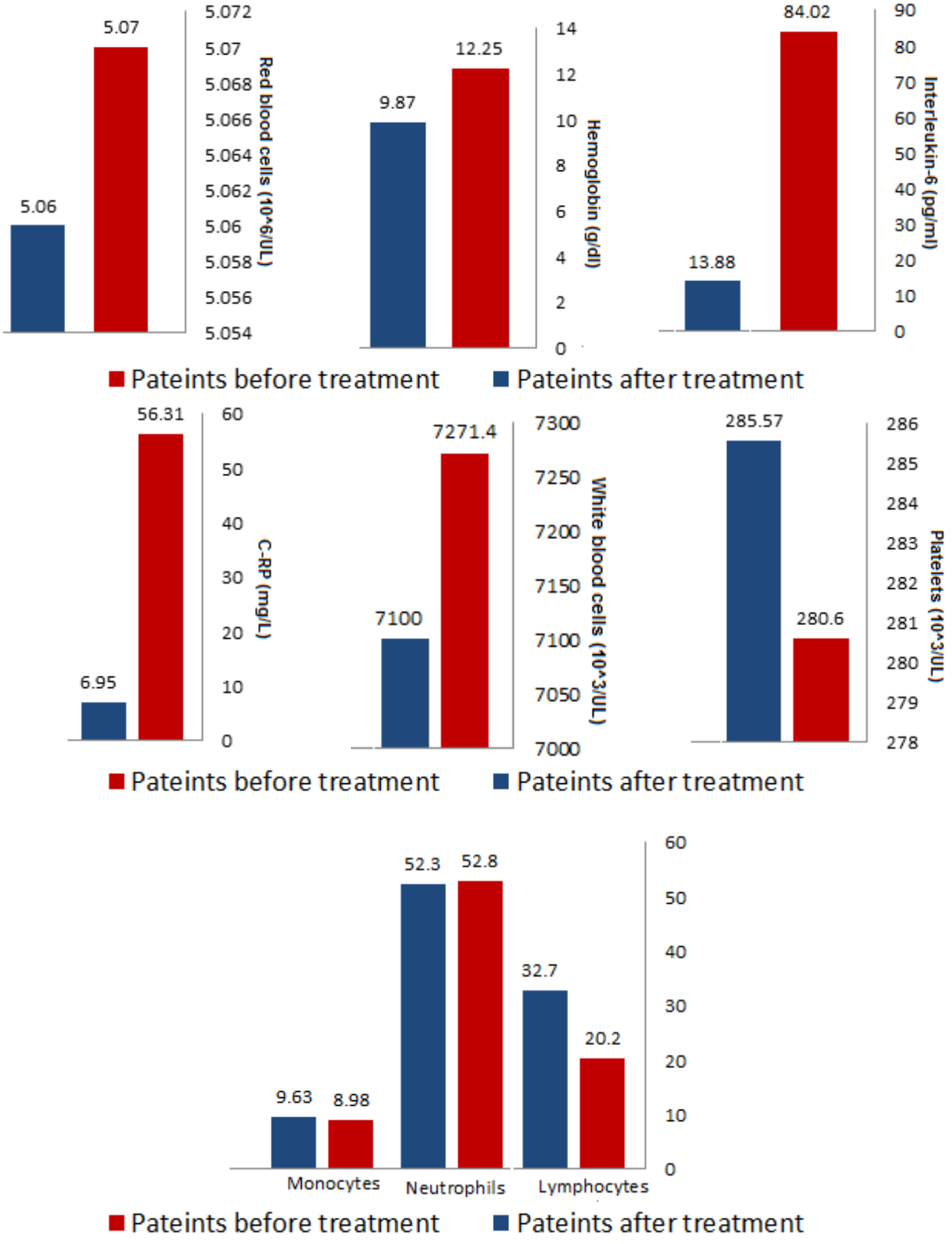

Figure 1: Show interleukin-6, C. reactive protein, and hematological parameter analysis of studied patients before and after treatment.

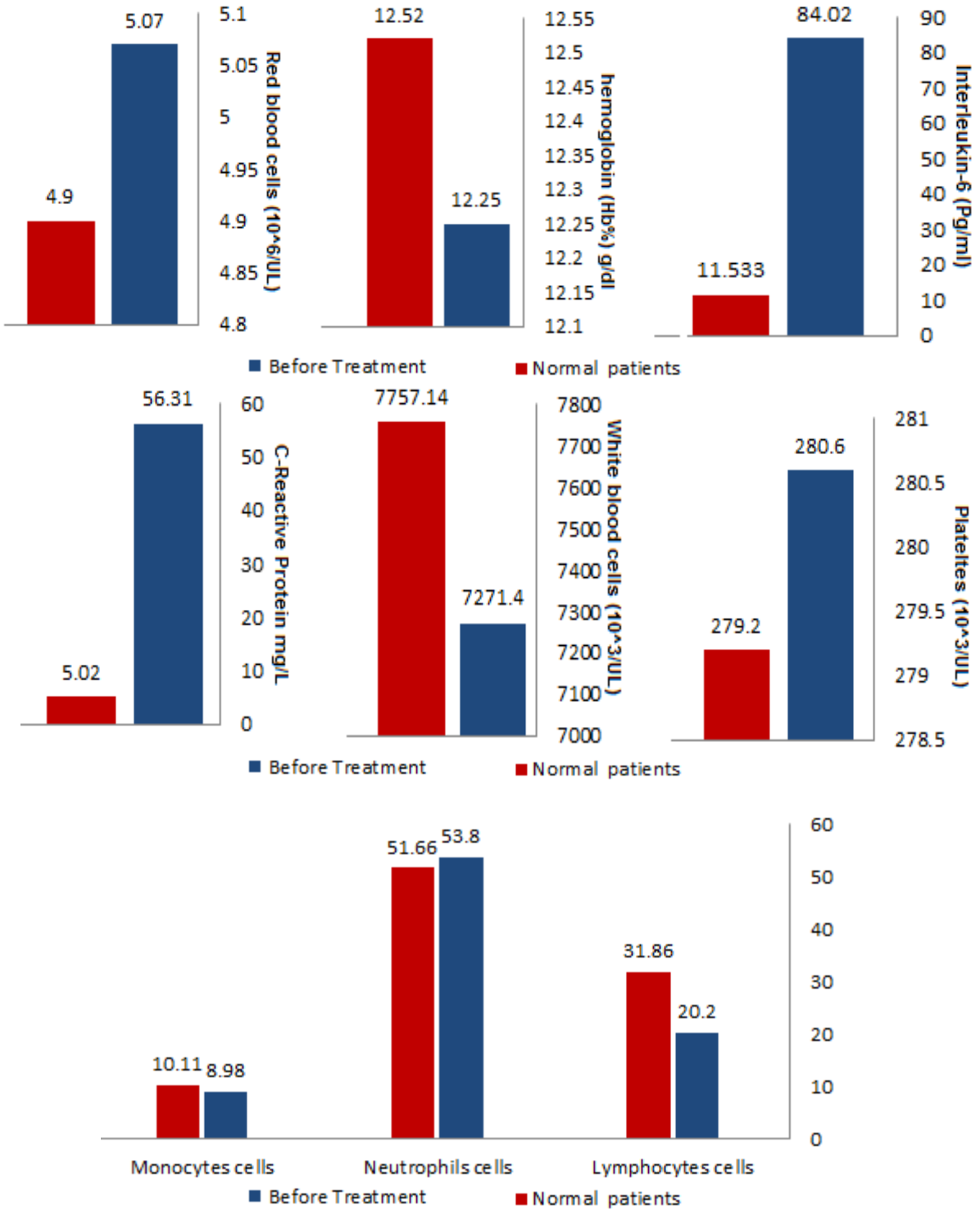

Figure 2: Show the study of interleukin-6, C-reactive protein, and hematological parameters in patients who were evaluated before therapy for COVID-19 infection and patients who do not have COVID-19 infection (normal patients).

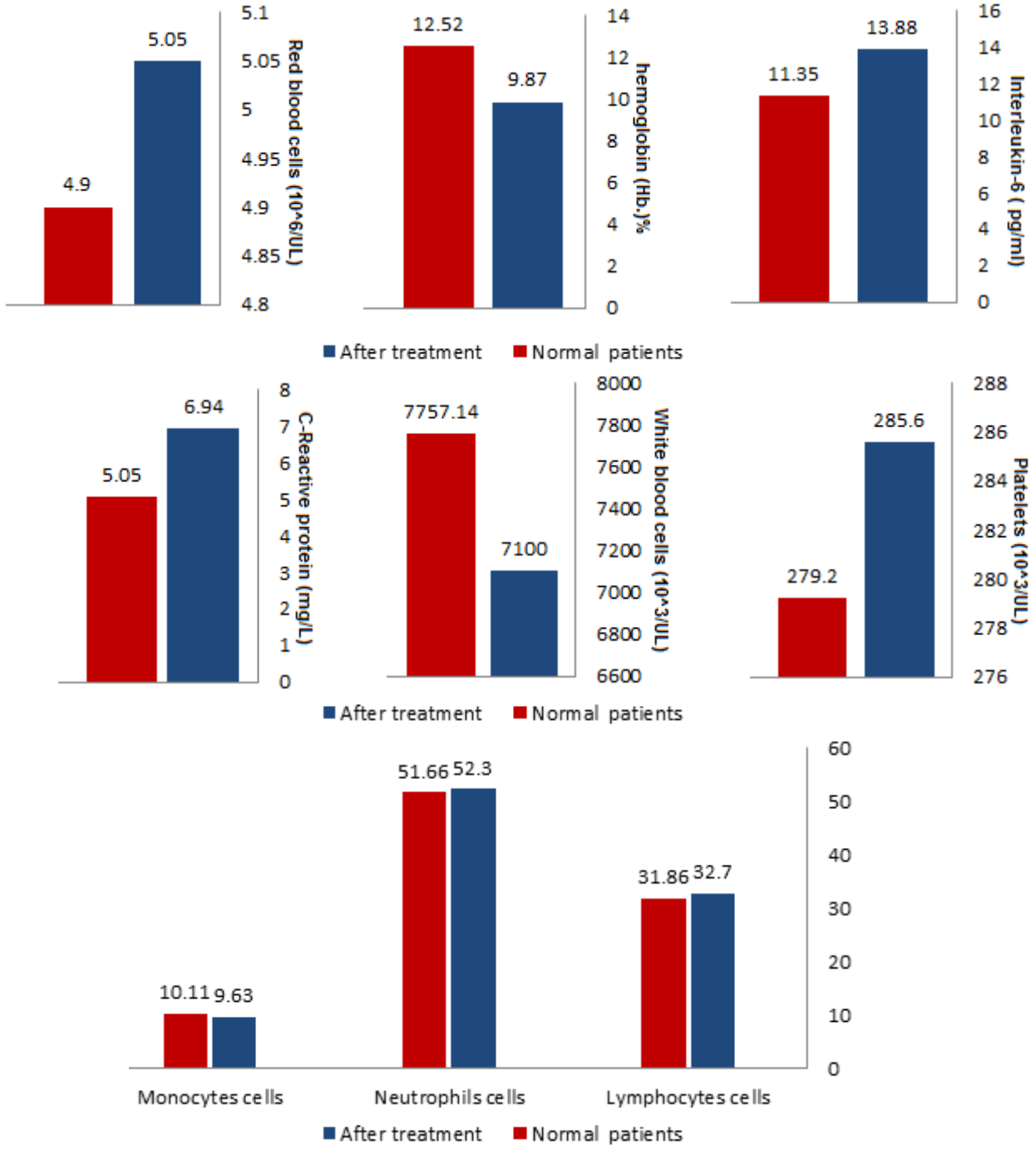

Figure 3: Show the study of interleukin-6, C-reactive protein, and hematological parameters in patients who were evaluated after therapy for COVID-19 infection and patients who do not have COVID-19 infection (normal patients).

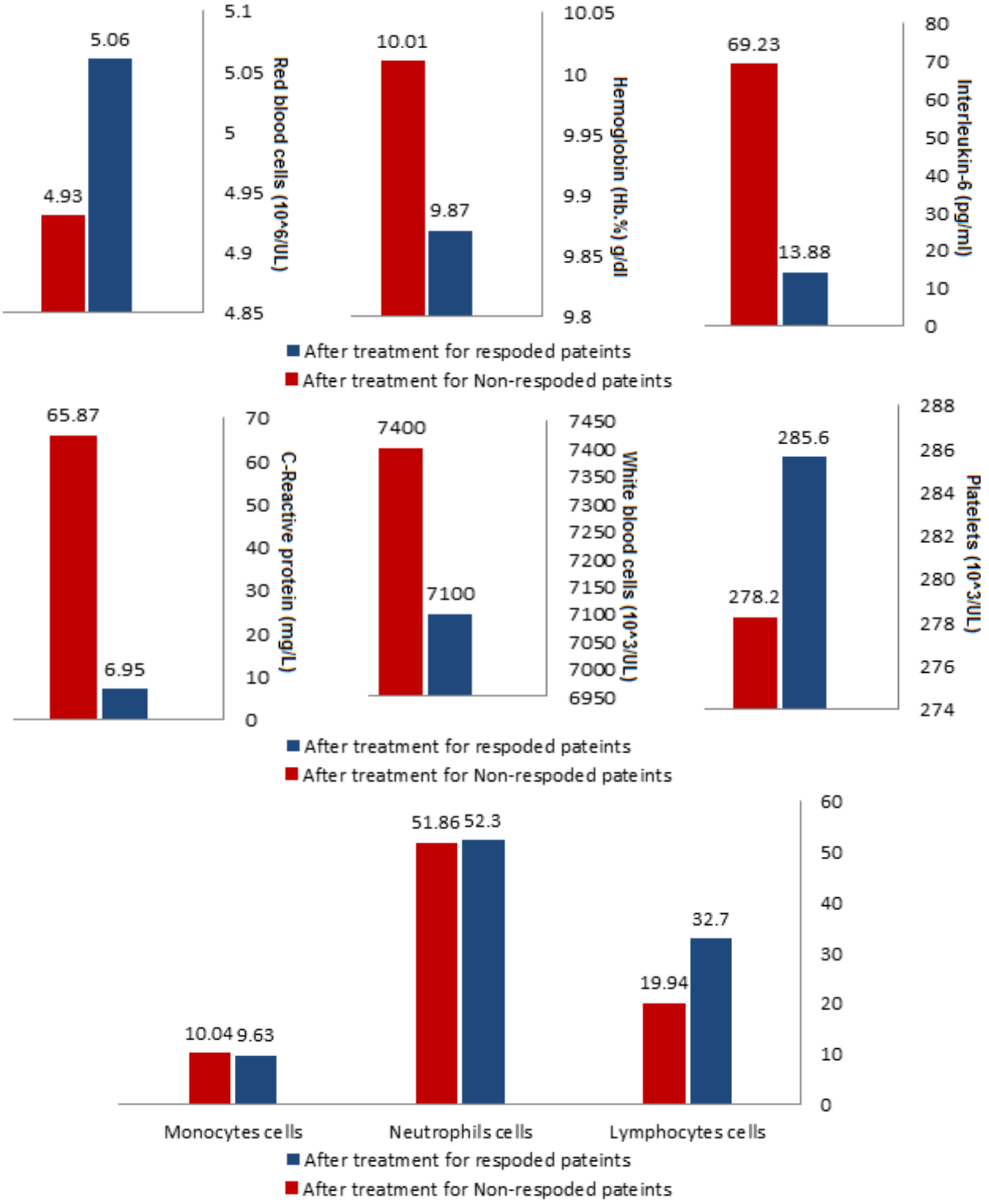

Figure 4: Show interleukin-6, C- reactive protein, and hematological parameters for patients with COVID-19 who responded to treatment and patients who did not.

Discussion

The initial clinical, biochemical, and demographic features of COVID-19 patients who were categorized as either non-severe or severe were investigated in this study. In patients with COVID-19 admitted to critical care units, there was a rise in the anti-inflammatory T-helper-2 (Th2) cytokines and higher levels of proinflammatory cytokines **[27]**. The percentage of severe cases corresponded with the risk of severity reported in the literature **[28]**. According to the current study, elderly patients were more likely to be severe and not recover. Patient age is linked to both a slower rate of illness onset and a greater death rate **[29]**.

The Global Health 50/50 study program gave an amazing overview of sex-disaggregated data from all over the world **[30]**. It was evident that although there were almost equal numbers of instances among men and women, the case fatality rate was higher in men. In our examination to discover COVID-19, we found that 660 patients (60 percent) had positive results and 440 cases (40 percent) had negative results. The virus causes symptoms like fever, coughing, and shortness of breath. SARS2 RNA RT-PCR was used to confirm these findings.

Randomly, 50 patients infected with COVID-19 were selected from 660 patients who were given nasal swabs and were moved to the intensive care unit to receive treatment followed by the Egyptian Ministry of Health. Immunological and chemical tests were done for patients before and after receiving treatment, and the response to treatment was confirmed by RNA SASRS2 by PCR. 50 people were selected without infection with COVID-19, and this was confirmed by PCR, and immunological and chemical tests were done for them.

The pathophysiology of COVID-19 is significantly impacted by the inflammatory response. Peripheral blood levels of the proinflammatory cytokine rose in the patients **[31]**. The National Health Commission of China (NHC) (trial version seven) reports that peripheral blood IL-6 levels rose after COVID-19 infection **[32]**. The level of the acute-phase inflammatory cytokine IL-6 in the serum indicates the severity of lung inflammation. **[33]**. Patients with severe COVID-19 had higher serum IL-6 levels than those with mild COVID-19. **[34]**. These findings corroborate the theory that lung damage from viral infection is caused by cytokine effects **[35]**.

One biomarker that can identify tissue injury, inflammation, and infection is CRP. Following acute inflammatory responses, the CRP level quickly increases **[36]**. It has been suggested that CRP is a helpful indicator for assessing the severity of COVID-19 **[37,38]**. Among hematological markers, lymphopenia is unquestionably connected with the severity of the disease; patients who died from COVID-19 had many fewer lymphocytes than those who survived. Indeed, the replacement of lymphocytes may be essential for healing **[39]**. Although their significance is still understood, other blood components, including platelets, neutrophils, eosinophils, white blood cells, and CD8 cell/ counts, were partially predictive of mild versus severe COVID-19. Research has demonstrated that the severity of a patient's illness is connected with higher levels of granulocyte colony-stimulating factor (G-CSF) in intensive care unit (ICU) patients **[39-41]**. Our immunological investigation of the interleukin-6 test revealed patients who had both COVID-19 infection and had not received treatment, as the results demonstrated a significant difference between before and after treatment, with the level of interleukin-6 increasing during COVID-19 infection and progressively decreasing with treatment until full recovery, as well as the CRP test. According to Table 4 and Figure 1 of our study, there is a significant difference between COVID-19 before and after therapy. Our study on blood cells revealed a significant difference in lymphocyte levels between patients with COVID-19 infections before and after treatment exposure. The level of lymphocytes decreases during COVID-19 infection (before

treatment) and progressively increases with treatment until full recovery (after treatment). Unlike the hemoglobin level percentage, our investigation demonstrated statistically significant variations between patients before and after treatment exposure, during COVID-19 infection, and after treatment. Whereas there are no significant differences in total white blood cells (WBCs), neutrophils, monocytes, red blood cells (RBCs), and blood platelets (PLTS).

In COVID-19 patients, IL-6 is one of the main mediators of inflammation and the viral cytokine storm **[42]**. Tocilizumab, an antibody that has been humanized and inhibits IL-6 receptors, has been shown in some studies to be effective in treating COVID-19 due to its ability to inhibit cytokine storms **[43]**. Our immunological study on interleukin-6 test showed patients with COVID-19 infections and before exposure to treatment, as the results showed that there is a significant difference in cases of infection (before treatment) and among patients who are not infected with COVID-19 (normal patients) where the level of interleukin-6 increases during infection with COVID-19, as well as the CRP test. Our study showed that there is a significant difference.

Our study on blood cells showed that in patients with COVID-19 infections and before exposure to treatment, there is a significant difference in lymphocyte cells in cases of infection (before treatment) and among patients who are not infected with COVID-19 (normal patients), where the level of lymphocyte cells decreases during COVID-19 infection. There are no significant differences in hemoglobin, red blood cells, total white blood cells, neutrophil cells, monocyte cells, or blood platelets. Our study on the interleukin-6 test showed patients with COVID-19 infections and after exposure to treatment, as the study showed that there is a significant difference in case of infection (after treatment) and among patients who are not infected with COVID-19 (normal patients). where there is a slight increase in the level of interleukin-6 during infection with COVID-19 after treatment, as well as the CRP test.

Our study on blood cells showed that in patients with COVID-19 infections and after exposure to treatment, there is a significant difference in hemoglobin levels in cases of infection (after treatment) and among patients who are not infected with COVID-19 (normal patients), where the level of hemoglobin decreases during COVID-19 infection and treatment. There are no significant differences in red blood cells, total white blood cells, neutrophil cells, lymphocyte cells, monocyte cells, or blood platelets.

Our investigation into the level of interleukin 6 in COVID-19-infected patients exposed to treatment revealed significant statistical differences between patients who responded to treatment and those who did not. Specifically, interleukin-6 levels significantly increased in non-responding patients while decreasing in treatment-responding patients, and the same was true for CRP levels. Additionally, our study demonstrated the level of lymphocyte cells in COVID-19-infected patients who were treated, and it also revealed statistical differences between patients who responded to treatment and those who did not; in the former group, lymphocyte cell levels significantly decreased, whereas, in the latter group, levels increased, While in hemoglobin, total white blood cells, neutrophils cells, monocyte cells, red blood cells, and blood platelets there are no significant.

Recommendation

Based on our research, we recommend to the Egyptian Ministry of Health that COVID-19 patients be closely observed, with pre-and post-treatment blood indicators, C-reactive protein, and interleukin-6 analyses necessary until full recovery.

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Ethics approval

The Ethics Committee at Al-Azhar University, Assiut Branch Faculty of Medicine, gave its approval for this study.

Conflict of interest

All authors declared that there were no conflicts of interest.

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References

1- **Channappanavar R. and Perlman S. (2017):** Pathogenic human coronavirus infections: causes and consequences of cytokine storm and immunopathology. *Semin Immunopathol.* 39:529–39.

2- **Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. (2020):** A novel coronavirus from patients with pneumonia in China, 2019. *N Engl J Med.* 382:727–33.

3- **Wiersinga WJ, Rhodes A, Cheng AC, Peacock SJ, Prescott HC. (2020):** Pathophysiology, transmission, diagnosis, and treatment of coronavirus disease 2019 (COVID-19): a review. *J Am Med Assoc.* 324:782–93.

4- Yameny, A. Short Communication: The COVID-19 JN.1 variant diagnosed in Egypt. *Journal of Medical and Life Science*, 2023; 5(4): 318-321. doi: 10.21608/jmals.2024.333814

5- **Channappanavar R, Perlman S (2017):** Pathogenic human coronavirus infections: causes and consequences of cytokine storm immunopathology. Semin Immunopathol 39(5):529–539

6- **Jesenak M, Brndiarova M, Urbancikova I, Rennerova Z, Vojtkova J, Bobcakova A et al (2020):** Immune parameters and COVID-19 infection - associations with clinical severity and disease prognosis. Front Cell Infect Microbiol 10:364

7- **Aziz M, Fatima R, Lee-Smith W, Assaly R (2020):** The association of low serum albumin level with severe COVID-19: a systematic review and meta-analysis. Crit Care 24:255

8- **Tanaka, T., Narazaki, M., and Kishimoto, T. (2016):** Immunotherapeutic implications of IL-6 blockade for cytokine storm. Immunotherapy.8 (8), 959–970.

9- **Tanaka, T., Narazaki, M., and Kishimoto, T. (2014):** IL-6 in inflammation, immunity, and disease. Cold Spring Harb. Perspect. Biol. 6 (10), a016295.

10- **Heinrich, P. C., Castell, J. V., and Andus, T. (1990):** Interleukin-6 and the acute phase response. Biochem. J. 265 (3), 621–636. doi:10.1042/bj2650621

11- **Yang M, Li CK, Li K, Hon KL, Ng MH, Chan PK, et al. (2004):** Hematological findings in SARS patients and possible mechanisms (review). Int J Mol Med.; 14:311–5.

12- **Chu H, Zhou J, Wong BH, Li C, Chan JF, Cheng ZS, et al. (2016):** Middle East respiratory syndrome coronavirus efficiently infects human primary T lymphocytes and activates the extrinsic and intrinsic apoptosis pathways. J Infect Dis.; 213:904–14.

13- **Channappanavar R, Zhao J, Perlman S. (2014):** T cell-mediated immune response to respiratory coronaviruses. Immunol Res.; 59:118–28.

14- **Qin C, Zhou L, Hu Z, Zhang S, Yang S, Tao Y, et al. (2020):** Dysregulation of immune response in patients with coronavirus 2019 (COVID-19) in Wuhan, China. Clin Infect Dis.; 71:762–8.

15- **Li D, Chen Y, Liu H, Jia Y, Li F, Wang W, et al. (2020):** Immune dysfunction leads to mortality and organ injury in patients with COVID-19 in China: insights from ERS-COVID-19 study. Signal Transduct Target Ther.;5:62.

16- **Nienhold, R.; Ciani, Y.; Koelzer, V.H.; Tzankov, A.; Haslbauer, J.D.; Menter, T.; Schwab, N.; Henkel, M.; Frank, A.; Zsikla, V.; et al. (2020):** Two distinct immunopathological profiles in autopsy lungs of COVID-19. Nat. Commun., 11, 5086.

17- **Mueller, A.A.; Tamura, T.; Crowley, C.P.; DeGrado, J.R.; Haider, H.; Jezmir, J.L.; Keras, G.; Penn, E.H.; Massaro, A.F.; Kim, E.Y. (2020):** Inflammatory Biomarker Trends Predict Respiratory Decline in COVID-19 Patients. Cell Rep. Med., 1, 100144.

18- **Parimoo, A.; Biswas, A.; Baitha, U.; Gupta, G.; Pandey, S.; Ranjan, P.; Gupta, V.; Barman Roy, D.; Prakash, B.; Wig, N. (2021):** Dynamics of Inflammatory Markers in Predicting Mortality in COVID-19. Cureus, 13, e19080.

19- **Smilowitz, N.R.; Kunichoff, D.; Garshick, M.; Shah, B.; Pillinger, M.; Hochman, J.S.; Berger, J.S. (2021):** C-reactive protein and clinical outcomes in patients with COVID-19. Eur. Heart J., 42, 2270–2279.

20- **Kayser, S.; Kunze, R.; Sheriff, A. (2021):** Selective C-reactive protein apheresis for Covid-19 patients suffering from organ damage. Ther. Apher. Dial., 25, 251–252.

21- **Pepys, M.B. (2021):** C-reactive protein predicts outcome in COVID-19: Is it also a therapeutic target? Eur. Heart J. 42, 2280–2283.

22- **Sheriff, A.; Schindler, R.; Vogt, B.; Abdel-Aty, H.; Unger, J.K.; Bock, C.; Gebauer, F.; Slagman, A.; Jerichow, T.; Mans, D.; et al. (2015):** Selective apheresis of C-reactive protein: A new therapeutic option in myocardial infarction? J. Clin. Apher., 30, 15–21.

23- **Ries, W.; Torzewski, J.; Heigl, F.; Pfluecke, C.; Kelle, S.; Darius, H.; Ince, H.; Mitzner, S.; Nordbeck, P.; Butter, C.; et al. (2021):** C-Reactive Protein Apheresis as Anti-inflammatory Therapy in Acute Myocardial Infarction: Results of the CAMI-1 Study. Front. Cardiovasc.Med., 8, 155.

24- **Torzweski, J.; Heigl, F.; Zimmermann, O.; Wagner, F.; Schumann, C.; Hettich, R.; Bock, C.; Kayser, S.; Sheriff, A. (2020):** First-in-man: Case report of Selective C-reactive Protein Apheresis in a Patient with SARS-CoV-2 Infection. Am. J. Case Rep., 21, e925020.

25- **Henry BM. (2020):** COVID-19, ECMO, and lymphopenia: a word of caution. Lancet Respir Med.

26- **Thirumalaisamy P. Velavana,B.: Christian G. Meyer.(2020):** Mild versus severe COVID-19: Laboratory markers., International Journal of Infectious Diseases . 95 304–307.

27- **Huang, C.; Wang, Y. and Li, X.(2020):** Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China, The Lancet , **395**(10223): 497-506.

28- **Hu Y, Sun J, Dai Z, Deng H, Li X, Huang Q et al (2020):** Prevalence and severity of corona virus disease 2019 (COVID-19): a systematic review and meta-analysis. J Clin Virol,127:104371.

29- **Wang L, He W, Yu X, Hu D, Bao M, Liu H et al (2020):** Coronavirus disease 2019 in elderly patients: characteristics and prognostic factors based on 4- week follow-up. J Inf Secur, 80(6):639–645.

30- Sex, gender and Covid-19. 2020. Global Health 5050.https://globalhealth5050.org/covid19/

31- **Ragab D, Salah Eldin H, Taeimah M, Khattab R, Salem R (2020):** The COVID-

19 cytokine storm; what we know so far. Front Immunol 11:1446

32- **Zhao M (2020):** Cytokine storm and immunomodulatory therapy in COVID-19: role of chloroquine and anti-IL-6 monoclonal antibodies. Int J Antimicrob Agents 55(6):105982

33- **de Brito RC, Lucena-Silva N, Torres LC, Luna CF, Correia JB, da Silva GA (2016):** The balance between the serum levels of IL-6 and IL-10 cytokines discriminates mild and severe acute pneumonia. BMC Pulm Med 16:170

34- **Aziz M, Fatima R, Assaly R (2020):** Elevated interleukin-6 and severe COVID-19: a meta-analysis. J Med Virol. https://doi.org/10.1002/jmv.25948

35- **Liu T, Zhang J, Yang Y, Ma H, Li Z, Zhang J et al (2020):** The role of interleukin-6 in monitoring severe case of coronavirus disease 2019. EMBO Mol Med 12(7): e12421

36- **Chen W, Zheng KI, Liu S, Yan Z, Xu C, Qiao Z (2020):** Plasma CRP level is positively associated with the severity of COVID-19. Ann Clin Microbiol Antimicrob 19:18

37- **Henry BM. (2020):** COVID-19, ECMO, and lymphopenia: a word of caution. Lancet Respir Med.

38- **Alabd, S., Yameny, A.** C-Reactive Protein as a Prognostic Indicator in COVID-19 mild infection Patients. *Journal of Medical and Life Science*, 2021; 3(2): 38-43. doi: 10.21608/jmals.2021.240126

39- **Thirumalaisamy P. Velavana,B.: Christian G. Meyer. (2020):** Mild versus severe COVID-19: Laboratory markers., International Journal of Infectious Diseases. 95 304–307.

40- **Yameny, A.** Association between thrombocytopenia and mild infection of COVID-19 patients. *Journal of Bioscience and Applied Research*, 2021; 7(3): 130-134. doi: 10.21608/jbaar.2021.200859

41- **Yameny, A.** Characteristics of peripheral Leukocyte in moderate infection of COVID-19. *Journal of Bioscience and Applied Research*, 2021; 7(4): 216-222. doi: 10.21608/jbaar.2021.251237

42- **Lee DW, Gardner R, Porter DL et al. (2014):** Current concepts in the diagnosis and management of cytokine release syndrome. *Blood* 124(2),188–195 (2014).

43- **Dholaria BR, Bachmeier CA, Locke F. (2019):** Mechanisms and management of chimeric antigen receptor T-cell therapy-related toxicities. *BioDrugs* 33(1), 45–60 (2019).