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# Original Article:
# Comparing Para-clinical and Laboratory Methods of Covid-19 Diagnosis in Iran

**Hamid Chegni[1*], Hedieh Haji Khodaverdi Khani[2], Zahra Nasiri[3], Fatemeh Eskandari Malayeri[4], Farhoodeh Ghaedrahmati[4], Mohammad Heidarvand[4]**

1. Department of Medical Laboratory Science, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
2. Department of Immunology, Faculty of Medical Science, Shahed University, Tehran, Iran
3. Convergent Technology Research Center, Tehran University, Tehran, Iran
4. Department of Immunology, Faculty of Medical Science, Isfahan University of medical sciences, Isfahan, Iran

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*** Corresponding author:**
Hamid Chegni, PhD.
**Address:** Department of Medical Laboratory Science, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
**E-mail:** h.chegini2010@yahoo.com

## Abstract

**Introduction:** Coronavirus Disease (COVID-19) caused by Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2) was first discovered in China in late 2019 and spread rapidly worldwide. This study aimed to correlate positive real time Reverse Transcriptase Polymerase Reaction (RT-PCR) results after one month of follow-up with laboratory findings of the same patients at hospital admission to predict clinical outcome and diagnosis.

**Materials and Methods:** We conducted a retrospective study on the laboratory findings of 299 adult patients suspected of COVID-19. Patients were admitted to hospital from March 21 to May 25, 2021 with final follow-up of one month for each patient. After one month of follow-up, 233 patients recovered; however, in 64 patients the symptoms worsened. For these patients RT-PCR was performed and some patients needed chest Computed Tomography (CT) imaging and were hospitalized. We extracted laboratory findings of these 64 patients and correlated the results of their RT-PCR with their laboratory findings.

**Results:** Based on our findings, severe cases are middle-aged adults (P=0.001) with lymphopenia (P<0.001), decreased levels of white blood cells (WBCs) (P<0.001), and platelets (P=0.007) count along with elevated COVID-19 IgG antibody (P=0.002) and Erythrocyte Sedimentation Rate (ESR) (P<0.001).

**Conclusion:** RT-PCR is not necessary at admission; instead, some routine hematology examinations and serological tests can predict the prognosis of COVID- 19 disease.

**Key words:** Covid-19, Laboratory findings, Diagnosis, Para-clinical

## 1. Introduction

In December 2019, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) -also called 2019 novel coronavirus, 2019-nCoV- the causative agent of a new outbreak, emerged in Wuhan, China and spread dramatically all over the world, becoming a health threat worldwide [1-3]. SARS-CoV-2 belongs to the Coronavirinae subfamily in the Coronaviridae family of the Nidovirales order [4]. CoVs are single-stranded positive RNA (+ssRNA) (~30 kb) which have at least four main structural proteins including spike (S), membrane (M), envelope (E) and nucleocapsid (N) proteins [5]. The S protein consists

of two subunits: The S1 subunit or bulb that contains a receptor-binding domain (RBD) which interacts with the Angiotensin II Converting Enzyme (ACE2) receptor on the membrane of host cell (such as type 2 alveolar cells). This interaction induces a conformational change in the S2 subunit or stalk of the S protein leading to cleavage of S1 and S2 that facilitates fusion of viral envelope with the host cell membranes. Therefore, it allows viral RNA to enter the cytoplasm of target cells [5-9]. Virus may reduce anti-viral interferon responses that lead to uncontrolled viral replication. The infiltration of neutrophils and monocytes/macrophages leads to overproduction of pro-inflammatory cytokines. The immunopathology of lung like pulmonary edema and pneumonia may be the result of the "cytokine storms". Specific Th1/Th17 may be stimulated, thus contributing to aggravate inflammatory responses. B cells/plasma cells are also activated and produce antibodies specific to SARS-CoV-2 that may assist in neutralizing viruses [10]. COVID-19 is transmitted to healthy individuals through the inhalation of respiratory droplets of the infected patients when coughing or sneezing, close personal contact, or contact with contaminated surfaces. The average incubation period before patients show symptoms is 2 to 14 days [11]. Most cases of COVID-19 (approximately 80%) present with asymptomatic or mild symptoms characterized by fever, cough, malaise, upper respiratory symptoms and with or without shortness of breath [12] while the rest (approximately 15-20%), which show significant abnormalities in laboratory findings, would develop severe form of the disease warranting hospitalization in intensive care units (ICU) [13, 14]. Organ dysfunction including shock, acute respiratory distress syndrome (ARDS), acute cardiac injury (ACI), and acute kidney injury (AKI) may occur in 5% of severe cases of COVID-19 [12, 13]. Older age, male gender, and co-morbidities such as cardiovascular diseases or hypertension as well as diabetes and obesity are known to be risk factors for severe COVID-19 [15, 16]. At present, real time reverse transcriptase polymerase reaction (RT-PCR) is the main diagnostic tool for detecting cases of SARS-CoV-2, collected from nasal and pharyngeal swabs and bronchoalveolar lavage (BAL) fluids. In addition, chest computed tomography (CT) imaging, some hematology examinations, and serologic immunoassays are used to complement the diagnosis [17, 18]. Clinical features, laboratory findings, and their relationship to outcome in COVID-19 patients can be crucial in management and timely diagnosis in order to prevent their spread as well as more effective control of disease progression. This study correlated patients with positive RT-PCR results after one month of follow-up with their laboratory findings both to predict clinical outcome and diagnosis and to see whether RT-PCR is a necessary test or not.

## 2. Materials and Methods

We carried out a retrospective study on the laboratory findings of 299 adult patients who were suspected of COVID-19. The participants were all outpatients aged between 7 and 85. Our inclusion criteria were symptoms including fever, dry cough, myalgia, fatigue, shortness of breath, and headache. All patients were in moderate and mild stages of the disease. They were all informed about the study and signed an informed consent. This study was approved by the Research Ethics Committee.

Blood samples were collected from each participant and routine blood test including white blood cell (WBC), lymphocyte (lymph), and platelet count (PLT) were performed on the blood samples on Sysmex KX-21 (Sysmex, Japan). Furthermore, COVID-19 IgM and IgG antibody titers in patients' serum based on the ELISA method, and inflammation-related parameters (C-reactive protein (CRP) based on latex agglutinasition test and erythrocyte sedimentation rate (ESR) based on automated modified Westergren) were assessed. After one month of follow-up, RT-PCR from nasopharyngeal and oropharyngeal samples and CT imaging were performed.

The results were presented as mean ± standard deviation (SD) or standard error (SE) range. Independent sample t-test was used to compare the laboratory findings between genders, positive and negative results of CRP, RT-PCR and CT imaging. ANOVA was employed to compare the laboratory findings between CRP positive and negative groups. In addition, Chi-square and Pearson correlation tests were used to compare genders in study groups and check correlation between laboratory findings, respectively. Statistical analyses were performed using SPSS software for Windows release 25.0 (SPSS Inc., IL, USA). Statistical significance was considered to be $P<0.05$.

## 3. Results

Our study included 299 suspected COVID-19 individuals. The mean age was 35.88 ± 12.53 (range: 7-85) years old, with 130 of the patients being female and 169 male. We first compared age and laboratory findings including WBC, lymph, PLT, ESR, COVID-19 IgM and IgG antibody titers between males and females. Table 1 shows the sex-specific laboratory findings of the 299 patients suspected of COVID-19. Among all of the parameters, PLT and ESR were significantly different between the

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**Table 1.** The sex-specific laboratory findings of the 299 patients with COVID-19

| Gender | | N | Mean±SD | SE | P |
|---|---|---|---|---|---|
| Age | Female | 130 | 37.52± 14.09 | 1.24 | 0.054 |
| | Male | 169 | 34.62± 11.05 | 0.85 | |
| WBC | Female | 130 | 6266.92± 2669.97 | 234.17 | 0.214 |
| | Male | 169 | 5922.49± 1903.81 | 146.45 | |
| Lymph | Female | 130 | 1893.08± 812.71 | 71.28 | 0.465 |
| | Male | 169 | 1957.99± 687.61 | 52.89 | |
| PLT | Female | 130 | 228846.15± 70182.98 | 6155.45 | <0.001 |
| | Male | 169 | 199396.45± 52991.04 | 4076.23 | |
| ESR | Female | 129 | 18.09± 14.88 | 1.31 | 0.029 |
| | Male | 169 | 14.36± 14.32 | 1.10 | |
| Covid IgM | Female | 130 | 0.56± 2.16 | 0.19 | 0.110 |
| | Male | 169 | 0.25± 0.23 | 0.02 | |
| Covid IgG | Female | 130 | 0.80± 1.68 | 0.15 | 0.140 |
| | Male | 169 | 0.53±1.41 | 0.11 | |

two groups (P<0.001 and P=0.029, respectively). Males had significantly decreased PLT counts compared to females while females had significantly increased ESR levels.

In addition, CRP test was performed for all of the suspected patients. Table 2 compares the laboratory parameters of suspected patients with positive and negative CRP. The result of CRP was positive in 76 cases and negative in 223 cases. Patients with positive CRP had significantly lower lymph and significantly higher ESR and COVID-19 IgG (P<0.001, P<0.001 and P=0.024, respectively) compared to the negative CRP group. CRP results based on three categories from one to three positive is shown in Table 3 (P-value 1 shows statistical difference among the CRP positive groups with negative CRP group, P-value 2 shows statistical difference among the two and three positive with one positive CRP group and P-value 3 shows statistical difference among the three positive with the two positive CRP group).

Pearson's correlation test was used to examine the correlation between age and laboratory findings and also between different laboratory findings. Correlations are shown in Table 4. Correlations were found between age, lymph (P=0.005) and COVID-19 IgG (P<0.001), between WBC and lymph (P<0.001), PLT (P<0.001) and COVID-19 IgG (P=0.001), between lymph and PLT (P<0.001), ESR (P<0.001) and COVID-19 IgM (P= 0.044), between PLT and COVID-19 IgM (P=0.007) and IgG (P=0.028) and between COVID-19 IgM and IgG (P=0.041).

After one month of follow-up, 233 patients recovered but in 64 patients the symptoms worsened so that some needed chest Computed Tomography (CT) imaging and were hospitalized. Table 5 shows correlations of positive and negative RT-PCR results after one month of follow-up with their laboratory findings. Patients with positive RT-PCR were significantly older (P=0.001) and had significantly lower WBC (P<0.001), lymph (P<0.001) and PLT count (P=0.007) and significantly higher ESR (P<0.001) and COVID-19 IgG (P=0.002) levels in serum.

Among positive RT-PCR patients, 28 cases were positive for chest CT imaging and 269 cases were negative. Table 6 shows correlations of patients with positive and negative CT imaging with their laboratory parameters. Patients with positive CT imaging were significantly older (P<0.001) and had significantly lower WBC (P<0.001), lymph (P<0.001) and PLT count (P=0.001) and significantly higher ESR (P<0.001).

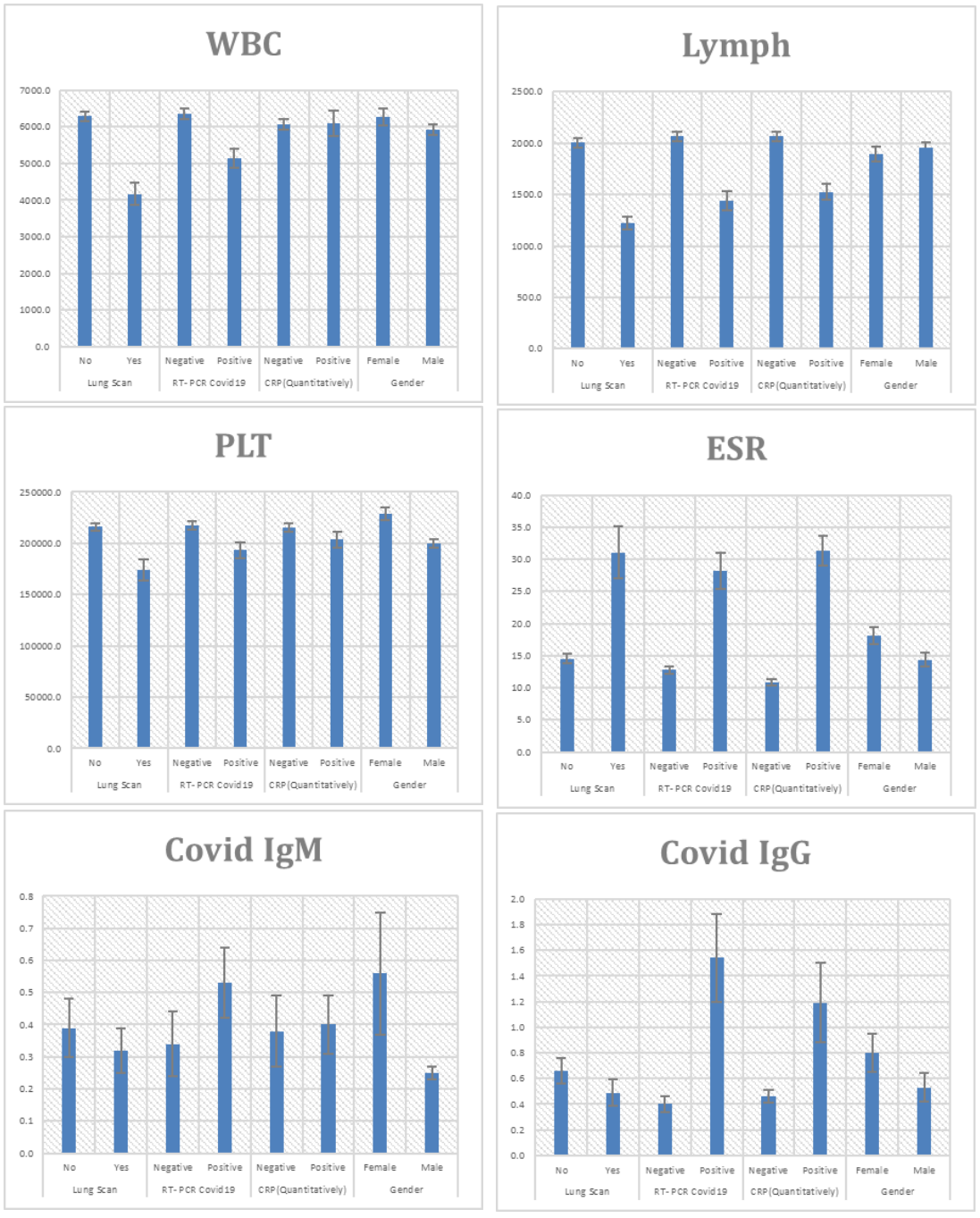


**Figure 1.** Comparison of laboratory parameters of suspected Covid-19 patients with Lung Scan, RT- PCR Covid-19, CRP, (Quantitatively) and Gender

Additionally, CRP, CT imaging and RT-PCR tests were also compared between genders. Out of 223 negative CRP patients, 94 were female and 129 were male, and out of 76 positive CRP patients, 36 were female and 40 were male. Regarding CRP, there was no significant difference between the two groups. After one month of follow-up, gender was also compared between positive CT imaging and RT-PCR patients. There was significant difference between the two groups in positive CT imaging patients. The results of gender comparison for CRP, RT-PCR and CT imaging are shown in Table 7 and Figure 1.

## 4. Discussion

In the face of the threat to human health posed by COVID-19, early laboratory evaluation and prognosis of the disease can pave the way for timely diagnosis, prevention of virus transmission, and effective control of

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**Table 2.** Compares the laboratory parameters of suspected Covid-19 patients with positive and negative CRP (P<0.001)

| CRP (Quantitatively) | | N | Mean±SD | SE | P |
|---|---|---|---|---|---|
| Age | Negative | 223 | 35.55±11.73 | 0.79 | 0.428 |
| | Positive | 76 | 36.87±14.65 | 1.68 | |
| WBC | Negative | 223 | 6064.57±1985.39 | 132.95 | 0.934 |
| | Positive | 76 | 6094.74±2971.24 | 340.82 | |
| Lymph (Absolute percentage of lymphocytes) | Negative | 223 | 2067.26±705.55 | 47.25 | <0.001 |
| | Positive | 76 | 1526.32±710.37 | 81.49 | |
| PLT | Negative | 223 | 215089.69±60656.70 | 4061.87 | 0.173 |
| | Positive | 76 | 203723.68±67997.57 | 7799.86 | |
| ESR | Negative | 223 | 10.83±6.84 | 0.46 | <0.001 |
| | Positive | 75 | 31.27±20.12 | 2.32 | |
| Covid IgM | Negative | 223 | 0.38±1.60 | 0.11 | 0.919 |
| | Positive | 76 | 0.40±0.80 | 0.09 | |
| Covid IgG | Negative | 223 | 0.46±0.75 | 0.05 | 0.024 |
| | Positive | 76 | 1.19±2.71 | 0.31 | |

disease progression. This study was conducted on 299 suspected patients of SARS-CoV-2 disease. Complete blood count (CBC) and inflammation-related parameters (ESR, CRP) are routine tests that are performed for COVID-19 patients. Yet, since false-negative result is observed in some cases when using RT-PCR method, additional tests such as serological tests can be used to detect COVID-19 infection [19]. In addition, in the absence of molecular diagnosis, serological tests are particularly important for the diagnosis of cases with mild to moderate disease [20].

Regarding gender, the platelet count was significantly lower in male group than that in female group and ESR was higher in female patients. As for CRP, there was no significant difference between the two groups. After one month of follow-up, gender was also compared between positive CT imaging and RT-PCR patients. There was a significant difference between the two groups in positive CT imaging patients. Differences in immune responses between females and males might be due to sex hormones and specific X-chromosome encoded genes [21].

During COVID-19 viral infection, IgM antibody is detected 3 days after infection which is the first line of humoral immunity defense; after that, high-affinity IgG antibody responses begin and play an essential role in long-term immune memory [22]. In our study higher levels of IgG was seen in severe RT-PCR positive patients but increase in IgM level was not statistically significant between negative and positive RT-PCR patients. It seems that severe cases of COVID-19 have stronger response in IgG antibodies.

Our study shows that middle-age is a risk factor for poor prognosis. The mean age of positive RT-PCR patients was 40.31. In agreement with the results of our study, the age group of 40–49 has been shown to be the most common age group affected by COVID-19 [23].

The initial WBC count was in direct correlation with severity of the disease. Reduction in platelet counts was also observed in our severe RT-PCR positive patients. It has been shown that thrombocytopenia is an important finding in severe COVID -19 patients [24]. The mechanism of thrombocytopenia in COVID-19 seems to be due to an autoimmune response against blood cells [25]. Other studies have indicated that patients with COVID-19 have significant thrombocytopenia and leucopenia [26]. In addition, our research showed that lymphopenia has occurred in severe patients, which is a major laboratory feature in COVID-19 patients. Research has demonstrated that more than 80% of critically ill COVID-19 patients have lymphopenia [27]. Several mechanisms

**Table 3.** CRP results of patients with Covid-19 based on three categories from one to three positive

| CRP (Qualiitatively) | | Valid N | Mean±SD | P 1 | P 2 | P 3 |
|---|---|---|---|---|---|---|
| Age | Neg | 223 | 35.55±11.73 | | | |
| | Pos (+) | 34 | 36.06±16.37 | 0.996 | | |
| | Pos (++) | 34 | 36.12±11.04 | 0.995 | >0.999 | |
| | Pos (+++) | 8 | 43.50±20.14 | 0.292 | 0.432 | 0.439 |
| WBC | Neg | 223 | 6064.57±1985.39 | | | |
| | Pos (+) | 34 | 6426.47±2906.03 | 0.821 | | |
| | Pos (++) | 34 | 6152.94±3222.20 | 0.997 | 0.959 | |
| | Pos (+++) | 8 | 4437.50±1462.81 | 0.191 | 0.116 | 0.218 |
| Lymph ((Absolute percentage of lymphocytes) | Neg | 223 | 2067.26±705.55 | | | |
| | Pos (+) | 34 | 1879.41±862.62 | 0.452 | | |
| | Pos (++) | 34 | 1205.88±367.58 | <0.001 | <0.001 | |
| | Pos (+++) | 8 | 1387.50±368.15 | 0.033 | 0.269 | 0.908 |
| PLT | Neg | 223 | 215089.69±60656.70 | | | |
| | Pos (+) | 34 | 220676.47±69047.51 | 0.962 | | |
| | Pos (++) | 34 | 190000.00±53208.00 | 0.129 | 0.180 | |
| | Pos (+++) | 8 | 190000.00±105788.20 | 0.678 | 0.594 | >0.999 |
| ESR | Neg | 223 | 10.83±6.84 | | | |
| | Pos (+) | 34 | 22.59±9.08 | <0.001 | | |
| | Pos (++) | 33 | 32.70±21.08 | <0.001 | <0.001 | |
| | Pos (+++) | 8 | 62.25±19.67 | <0.001 | <0.001 | <0.001 |
| Covid IgM | Neg | 223 | 0.38±1.60 | | | |
| | Pos (+) | 34 | 0.52±1.14 | 0.955 | | |
| | Pos (++) | 34 | 0.32±0.35 | 0.995 | 0.940 | |
| | Pos (+++) | 8 | 0.25±0.16 | 0.994 | 0.965 | 0.999 |
| Covid IgG | Neg | 223 | 0.46±0.75 | | | |
| | Pos (+) | 34 | 1.33±2.85 | 0.010 | | |
| | Pos (++) | 34 | 1.16±2.89 | 0.059 | 0.968 | |
| | Pos (+++) | 8 | 0.66±0.71 | 0.984 | 0.667 | 0.829 |

have been reported as for depletion of lymphocytes. These mechanisms include direct infection and killing of lymphocytes by the virus, destruction of lymphatic organs, and production of metabolic molecules produced by metabolic disorders that can kill lymphocytes [28].

In our study, CT imaging severity had positive correlation with middle-age, low WBC, lymph and PLT count. Also, elevated levels of ESR were related to the disease severity and clinical outcome.

**Table 4.** Correlation between age and laboratory findings in patients with Covid-19

| Variables | | Age | WBC | Lymph | PLT | ESR | Covid IgM | Covid IgG |
|---|---|---|---|---|---|---|---|---|
| Age | r | | -0.106 | -0.162** | -0.063 | 0.098 | 0.031 | 0.207** |
| | P | | 0.066 | 0.005 | 0.280 | 0.092 | 0.595 | <0.001 |
| WBC | r | -0.106 | | 0.412** | 0.361** | -0.017 | 0.068 | 0.195** |
| | P | 0.066 | | <0.001 | <0.001 | 0.765 | 0.242 | 0.001 |
| Lymph | r | -0.162** | 0.412** | | 0.307** | -0.302** | 0.116* | -0.037 |
| | P | 0.005 | <0.001 | | <0.001 | <0.001 | 0.044 | 0.525 |
| PLT | r | -0.063 | 0.361** | 0.307** | | -0.007 | 0.156** | 0.127* |
| | P | 0.280 | <0.001 | <0.001 | | 0.902 | 0.007 | 0.028 |
| ESR | r | 0.098 | -0.017 | -0.302** | -0.007 | | 0.019 | 0.086 |
| | P | 0.092 | 0.765 | <0.001 | 0.902 | | 0.738 | 0.140 |
| Covid IgM | r | 0.031 | 0.068 | 0.116* | 0.156** | 0.019 | | 0.118* |
| | P | 0.595 | 0.242 | 0.044 | 0.007 | 0.738 | | 0.041 |
| Covid IgG | r | 0.207** | 0.195** | -0.037 | 0.127* | 0.086 | 0.118* | |
| | P | <0.001 | 0.001 | 0.525 | 0.028 | 0.140 | 0.041 | |

**Table 5.** Correlations of Covid-19 patients with positive and negative RT-PCR results after one month of follow-up with their laboratory findings

| RT- PCR Covid-19 | | N | Mean±SD | SE | P |
|---|---|---|---|---|---|
| Age | Negative | 233 | 34.70± 12.59 | 0.83 | 0.001 |
| | Positive | 64 | 40.31± 11.51 | 1.44 | |
| WBC | Negative | 233 | 6356.65± 2252.67 | 147.58 | <0.001 |
| | Positive | 64 | 5131.25± 2055.41 | 256.93 | |
| Lymph | Negative | 233 | 2065.24± 690.42 | 45.23 | <0.001 |
| | Positive | 64 | 1435.94± 739.66 | 92.46 | |
| PLT | Negative | 233 | 217193.13± 62197.07 | 4074.67 | 0.007 |
| | Positive | 64 | 193359.38± 62227.79 | 7778.47 | |
| ESR | Negative | 233 | 12.76± 9.59 | 0.63 | <0.001 |
| | Positive | 63 | 28.17± 22.16 | 2.79 | |
| Covid IgM | Negative | 233 | 0.34± 1.56 | 0.10 | 0.360 |
| | Positive | 64 | 0.53± 0.90 | 0.11 | |
| Covid IgG | Negative | 233 | 0.40± 0.85 | 0.06 | 0.002 |
| | Positive | 64 | 1.54± 2.74 | 0.34 | |

**Table 6.** Correlations of Covid-19 patients with positive and negative CT imaging with their laboratory parameters

| Lung Scan | | N | Mean±SD | SE | P |
|---|---|---|---|---|---|
| Age | No | 269 | 34.92± 12.51 | 0.76 | <0.001 |
| | Yes | 28 | 45.43± 8.57 | 1.62 | |
| WBC | No | 269 | 6292.57± 2227.38 | 135.81 | <0.001 |
| | Yes | 28 | 4171.43± 1668.19 | 315.26 | |
| Lymph | No | 269 | 2002.97± 739.60 | 45.09 | <0.001 |
| | Yes | 28 | 1225.00± 332.92 | 62.92 | |
| PLT | No | 269 | 215970.26± 62457.02 | 3808.07 | 0.001 |
| | Yes | 28 | 174464.29± 54717.11 | 10340.56 | |
| ESR | No | 269 | 14.54± 12.97 | 0.79 | <0.001 |
| | Yes | 27 | 31.07± 21.30 | 4.10 | |
| Covid IgM | No | 269 | 0.39± 1.52 | 0.09 | 0.818 |
| | Yes | 28 | 0.32± 0.37 | 0.07 | |
| Covid IgG | No | 269 | 0.66± 1.61 | 0.10 | 0.563 |
| | Yes | 28 | 0.49± 0.52 | 0.10 | |

**Table 7.** The results of the sex comparison for CRP, RT-PCR and CT imaging in patients with Covid-19

| Gender n | | No. (%) | | P |
|---|---|---|---|---|
| | | Female | Male | |
| CRP (Qualitatively) | Neg | 94(72.3) | 129(76.3) | 0.884 |
| | Pos (+) | 16(12.3) | 18(10.7) | |
| | Pos (++) | 16(12.3) | 18(10.7) | |
| | Pos (+++) | 4(3.1) | 4(2.4) | |
| CRP (Quantitatively) | Negative | 94(72.3) | 129(76.3) | 0.428 |
| | Positive | 36(27.7) | 40(23.7) | |
| Lung Scan | No | 112(86.2) | 157(94.0) | 0.021 |
| | Yes | 18(13.8) | 10(6.0) | |
| RT- PCR Covid-19 | Negative | 96(73.8) | 137(82.0) | 0.089 |
| | Positive | 34(26.2) | 30(18.0) | |

We also found that an increase in CRP is correlated with low lymph count and elevated levels of ESR and COVID-19 IgG. CRP indicates infection, so higher levels are expected in the patients. Increase in the level of CRP as well as the extent of the increase might be associated with the disease progression and COVID-19 severity [29].

Correlations were found between age and lymph and COVID-19 IgG, between WBC and lymph, PLT and COVID-19 IgG, between lymph with PLT, ESR and COVID-19 IgM, between PLT and COVID-19 IgM and IgG and between COVID-19 IgM and IgG.

## Conclusion

The results of our study adduce the argument that the main features of laboratory findings in COVID-19 patients are low WBC, lymph, and PLT counts and elevated levels of ESR and COVID-19 IgG, which are related to severity of the disease. Thus, they might be accurate enough to predict severe cases of COVID-19.

## Ethical Considerations

### Compliance with ethical guidelines

This study was approved by Shahid Beheshti University Medical Science Ethics Committee (Code: IR.SBMU.RETECH.REC.1399.158).

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### Author's contributions

All authors have equally contributed to preparation of this article

### Conflict of interest

The authors declare no conflict of interest.

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