Sapporo Medical Journal
Volume 54, Issue 09, September, 2020

Immunological Parameters in Early Detection of COVID-19 Patients with Positive RT-PCR and Evaluation of the Possible Correlation between Immunological Parameters in Jordan.

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Abstract— Background: The worldwide pandemic of Coronavirus disease 2019 emerged in December 2019 in Wuhan, China. Early identification of immunological biomarkers isa critical step for the disease diagnosis and progression in pursuance of classifying high-risk patients within the dearth of relevant information. Material and method: In a retrospective study, the laboratory finding of 98 COVID19 positively diagnosed patientswith ages ranged from 9 up to 77 years and administered to Prince Hamza Hospital, Amman, Jordan were used in the study. We have adopted the immunological parameters of WBCs, neutrophils, monocyte, basophils, D-dimer,CRP,and ESR as markers for anticipated diagnostic indicators. Results: Laboratory findings of COVID-19 showed that total WBCs might not be affected with SARS-CoV-2 in the initial phase of the disease. While the lymphocyte, eosinophil,basophil and CRP gave a primary indication about the COVID-19 infection through lymphopenia, eosinopenia, basopenia and an increase in the CRP value.The results of total WBC give a highly significant positive correlation with neutrophils ($p<0.05$), negative correlation with eosinophil and ESR, and significant positive correlation with monocyte and CRP ($p<0.05$). The age of the patient gave a negative correlation with lymphocyte, a highly significant negative correlation with eosinophil ($p<0.01$), and a significant positive correlation with ESR and CRP ($p<0.05$). Furthermore, The ESR gave a significant positive correlation with age and CRP ($p<0.05$), negative correlation with total WBCs, neutrophils, monocyte, eosinophil, lymphocyte, and D-dimer. Finally, the D-dimer results gave negative correlations with eosinophil, monocyte, lymphocyte, and ESR. Conclusion: We can summarize from this study that COVID19 patients show lymphopenia, eosinopenia, basopenia and elevations in CRP levels. The increase in age harms the immunological parameters for COVID 19 patients regardless of gender.

Keywords: COVID19, Corona Virus, Lymphopenia, D-dimer, Jordan**.**

Introduction

In the year 2019, we have embedded in the microbiology and pathology books a new causative agent forthesevere acute respiratory syndrome. The disease attacked globally and attenuated modern societyon the economic, health, and social levels. The attacks were classified as a pandemic by the WHO. Anovel coronavirus named as Coronavirus Disease 2019 (COVID19) was behind these episodes[1, 2]. The rate of incidence among the infected people was increasing daily bases on a logarithmic scale. This dramatic increase in the number of infected people with a dearth in the knowledge about the epidemiology of the disease and no recommended guidelines for therapy have exhausted the health care system in some countries and more specifically lead to a health care system collapse in others. Coronavirus infectionshave affected more than 200 countries and regions around the world in the 2020 year[3].

COVID 2019 was firstly detected in December 2019 in Wuhan, China, arising from a zoonotic virus [1].At the beginning of 2020, the Chinese Center for Disease Control and Prevention reported the isolation of a novel coronavirus virus from a patient through a direct throat swab. In February 2020 the

novel coronavirus was entitled as severe acute respiratory syndrome-related coronavirus-2 (SARS-Cov-2) by the World Health Organization (WHO) [4]. SARS-CoV-2 shares a genetic homology of 79% to severe acute respiratory syndrome and 51.8% to the Middle East Respiratory Syndrome coronavirus and belongs to the genus β coronavirus of the coronavirus family [5].

As a zoonotic disease coronavirus was firstly transmitted from bats to humans. The virus is a positive-strand enveloped RNA with trans-infectivity between humankind. SARS-CoV-2 is transmitted by the direct contact of the infected patient and health individual's patients; the route of transmission is reported through respiratory droplets and aerosols [5]. Clinical manifestations of fever, cough, shortness of breath, and fatigue are common between the infected patients but the severity is varied according to genetic variations and the medical history[6]. After that, symptoms develop in some patients to dyspnea and pneumonia that require more management in the intensive care units to avoid any unexpected respiratory complications that may lead to death [7]. 67.4–88.0% of cases of COVID-19 were diagnosed by chest tomography (CT), indicating that pneumonia is the most common manifestation of the disease [3]. However, there are no main specific symptoms to diagnose SARS-CoV-2 patients, and until now just the detection of the viral genome using the polymerase chain reaction (PCR) analysis is the usable way to give positive infection with COVID 19 [8].

The immune system plays an important role in the response to COVID19 with significant differences among severe and non-severe patients [9]. The host immune system is essential for the resolution of COVID-19 infection, immune response wasconsisting of two phases: the first phase started during the period of incubation and this phase considered as non-severe phase, during this phase an adaptive immune response is obligatory to control viral proliferation, to eliminate the virus, and to prevent disease progression. The second phase of COVID-19 infections which is an important cause of death in certain patients the cytokine concentrations increase and release to the circulation with the aim of cell signaling (so-called cytokine storm) then promotes the progression of the disease to severe status and many organ damages[10].

Immune parameters could help to understand better the dynamics of immune system activation and probably to select the appropriate prognostic markers concerning disease outcomes and expected complications. Moreover, immune support treatment could be another possible step in the complex management of these patients. On the other hand, it is not clear whether the observed changes in immune parameters are a direct consequence of COVID-19 or are predisposing factors for this infection and its severe course. Several questions regarding the immune response and its dynamics remain unresolved and the ongoing studies should bring the answers.

Therefore, one of the most factor related to the severity of the Middle East respiratory syndrome coronavirus (MERS-CoV2) disease is the immunological change in the white blood cells. Also, previous study has addressed the difference of baseline leukocyte counts between the clinical stages in COVID-19 patients [11].

Immunological biomarkers are particularly important, as immunopathology has been suggested as a primary driver of morbidity and mortality with COVID-19. Lymphopenia is the most frequently described prognostic marker in COVID-19 and it appears to predict morbidity and mortality even at early stages [12]. The most findings across many studies were elevated levels of CRP and D-dimer, as well as

decreased lymphocyte counts [11, 13]. Thrombocytopenia and elevated D-dimer levels may be indicative of coagulopathies in COVID-19 patients with important therapeutic implications [14].

Jordan is one of the pandemic countries with COVID19. Until 14 September 2020, the number of cases reaches to 3314 patients and 24 death case [15]. Our goal in this study is to ask whether the inflammatory and immune responses to Middle East respiratory syndrome coronavirus (MERS-CoV2) infection, especially in the early stage of infection, are indeed dynamic in order to guide hypothesis and design of studies to address disease pathogenesis and therapeutic interventions. The present study aimed to determine the clinical laboratory immunological biomarkers that will allow earlypredict and diagnosis of COVID 19 patients and evaluation of possible correlation between immunological parameterin Jordan, because there is no sufficient data that characterize the immunological parameter level between low, moderate and sever cases in early stage of infection.

Material and methods:

Data collection

We have conducted a retrospective study for 98 hospitalized patients in the Prince Hamza hospital (Amman, Jordan)between January 2020, and August 2020. Patients were administered tothe hospital after been diagnosed as COVID-19 Positive patients. The diagnosis criteria included pathological changes in the pulmonary structure diagnosed by MRI and confirmed by Real-Time PCR using novel coronavirus 2019-nCoV nucleic acid detection kit (fluorescent PCR method). The admission to the hospital in an isolation room with pulmonary distress and high fever. Complete analytical data of patients were collected from the Department of Laboratory Medicine.The immunological parameters of complete blood counts (CBC), Erythrocyte sedimentation rate(ESR), C- reactive protein (CRP), and D-Dimerwere collected for further investigations. The immunological tests were exposed forstatistical analysis for the correlation between the Immunological parameters and the clinical manifestations.

Statistical analysis

In this study, SPSS statistics software (version 19.0) was used for statistical data analysis and Graph Pad Prism software (version 8.0.2) was used for mapping. And other demographic and clinical characteristics were expressed in frequency and percentage. The correlation was done by using the Spearman correlation test, the statically significant $p<0.05$, and highly significant $p<0.01$.

Exclusion criteria

1- Females who are pregnant.
2- Any prior treatment against any other viral disease.
3- Autoimmune disease.
4- Individuals with any inflammatory condition.

Ethical Consideration

This study abidesby the Declaration of Helsinki (DOH). All obligatory ethical principles for medical research involving human subjects were found. The human subjects' confidentiality and rights were preserved throughout the study. All electronic laboratory tests were saved and subject identity remained classified. The anonymity and confidentiality of the subject identity and information were preserved

Result

Immunological biomarkers are requiredfor the primary diagnosesof patients as well as declaring the tendency for a good progression. The primary indicators of total white blood cell (WBC), differentials WBC count, CRP, ESR, and D-dimer parameters were reported to be associated with COVID-19infections [2].

The results of total WBCs, neutrophils, monocyte, D-dimer and ESRdid not show significant changes in SARS-CoV-2 initial phase of the disease. While the lymphocyte, eosinophil, basophils and CRP have given a significant primary indication about the viral infection through lemphopenia, eosinopenia and increase in the CRP value.

Laboratory findings of 98(32 females and 66 male patients of with COVID-19 are shown in (Table 1). The gender of COVID-19 patients did not show a significant difference in the immunological parameters as there was similar levels in the laboratory results in males and females. Total WBC count for the patients showed 5.1% leukopenia, 81.6% within the normal range and 13.3% leukocytosis. The differential count for neutrophils depicted 16.3% of patients with neutropenia, 69.4% within the normal range and 14.3% nutrocytosis. On the other hand, the results of basophils count demonstrate 35.7% basopenia and 64.3 within the normal range. Furthermore, majority of patients showed eosinopenia (83.7%) while only 11.2 within the normal range and 5.1% above the normal level. Also the results show that monocyte count gave 1% lower than the normal range, 88.8 within the normal range and 10.2 higher than the normal range. The lymphocyte showed heterogenous response 33% were lymphopenia 45% were within normal range and 22% showed lymphocytosis. Furthermore, the results of ESR and D-dimer (86.7%, 75.5% normal and 13.3%, 24.5% higher than normal value) respectively. Finally, CRP give a result 54.1% higher than normal and 45.9% normal value.Our results depicted a wide variability between laboratory marker and no certain pattern was observed.

Table 1: Frequency Table represents the frequency, percent, valid percent and cumulative represent laboratory findings of 98 (32 females and 66 male patients of with COVID-19).

		Frequency	Percent	Valid Percent	Cumulative Percent
WBCs	Below normal	5	5.1	5.1	5.1
	Normal	80	81.6	81.6	86.7
	Above normal	13	13.3	13.3	100
	Total	98	100	100	
Neutrophil	Below normal	16	16.3	16.3	16.3
	Normal	68	69.4	69.4	85.7
	Above normal	14	14.3	14.3	100
	Total	98	100	100	
Basophil	Below normal	35	35.7	35.7	35.7
	Normal	63	64.3	64.3	100
	Total	98	100	100	

Eosinophil	Below normal	82	83.7	83.7	83.7
	Normal	11	11.2	11.2	94.9
	Above normal	5	5.1	5.1	100
	Total	98	100	100	
Lymphocyte	Below normal	33	33.7	33.7	33.7
	Normal	45	45.9	45.9	79.6
	Above normal	20	20.4	20.4	100
	Total	98	100	100	
Monocyte	Below normal	1	1	1	1
	Normal	87	88.8	88.8	89.8
	Above normal	10	10.2	10.2	100
	Total	98	100	100	
ESR	Normal	85	86.7	86.7	86.7
	Above normal	13	13.3	13.3	100
	Total	98	100	100	
CRP	above normal	53	54.1	54.1	54.1
	Normal	45	45.9	45.9	100
	Total	98	100	100	
D-dimer	Normal	74	75.5	75.5	75.5
	Above normal	24	24.5	24.5	100
	Total	98	100	100	

A significant correlation was demonstrated between different parameters and the patient results. Such changes could be used as primary detection criteriafor COVID-19 patients as depicted in (Table 2). The age of patients ranged from (9-77 years) with a mean of 39.35 ± 1.58 and a median of 37. In grouping to age a negative correlation with lymphocyte ($p<0.05$) and eosinophil ($p<0.01$)was demonstrated, whereas,a significant positive correlation with ESR and CRP ($p<0.05$) has been detected. The total WBC gave high significant positive correlation with neutrophils,monocyte, and CRP ($p<0.05$), while there was a negative correlation with eosinophil and ESR.

		Age	WBC	Neutrophil	Basophil	Eosinophil	Monocyte	Lymphocyte	ESR	CRP	D DIMER
Age code	person Correlation	1	0.147	0.188	0.078	-0.168	0.011	-.336**	.246*	.417**	0.188
	Sig. (2-tailed)		0.150	0.064	0.443	0.098	0.913	0.001	0.015	0.000	0.063
	N	98	98	98	98	98	98	98	98	98	98
WBC	Person Correlation	0.147	1	.709**	0.145	-0.033	.246*	0.008	-0.076	.211*	0.059
	Sig. (2-tailed)	0.150		0.000	0.155	0.745	0.015	0.936	0.458	0.037	0.566
	N	98	98	98	98	98	98	98	98	98	98
Neutrophils	Person Correlation	0.188	.709**	1	.204*	0.051	0.182	-0.027	-0.040	.219*	0.064
	Sig. (2-tailed)	0.064	0.000		0.044	0.620	0.072	0.792	0.696	0.030	0.532
	N	98	98	98	98	98	98	98	98	98	98
Basophils	Person Correlation	0.078	0.145	.204*	1	0.102	.212*	.257*	0.103	0.046	0.127
	Sig. (2-tailed)	0.443	0.155	0.044		0.316	0.036	0.011	0.312	0.654	0.211
	N	98	98	98	98	98	98	98	98	98	98
Eosinophil	Person Correlation	-0.168	-0.033	0.051	0.102	1	0.004	.247*	-0.103	-0.065	-0.052
	Sig. (2-tailed)	0.098	0.745	0.620	0.316		0.966	0.014	0.311	0.527	0.610
	N	98	98	98	98	98	98	98	98	98	98
Monocyte	Person Correlation	0.011	.246*	0.182	.212*	0.004	1	0.005	-0.018	0.055	-0.015
	Sig. (2-tailed)	0.913	0.015	0.072	0.036	0.966		0.963	0.860	0.590	0.883
	N	98	98	98	98	98	98	98	98	98	98
Lymphocyte	Person Correlation	-.336**	0.008	-0.027	.257*	.247*	0.005	1	-0.018	-0.173	-0.063

	Sig. (2-tailed)	0.001	0.936	0.792	0.011	0.014	0.963		0.860	0.089	0.536
	N	98	98	98	98	98	98	98	98	98	98
ESR	Person Correlation	.246*	-0.076	-0.040	0.103	-0.103	-0.018	-0.018	1	.304**	-0.013
	Sig. (2-tailed)	0.015	0.458	0.696	0.312	0.311	0.860	0.860		0.002	0.900
	N	98	98	98	98	98	98	98	98	98	98
CRP	Person Correlation	.417**	.211*	.219*	0.046	-0.065	0.055	-0.173	.304**	1	0.094
	Sig. (2-tailed)	0.000	0.037	0.030	0.654	0.527	0.590	0.089	0.002		0.356
	N	98	98	98	98	98	98	98	98	98	98
D_DIMER	Person Correlation	0.188	0.059	0.064	0.127	-0.052	-0.015	-0.063	-0.013	0.094	1
	Sig. (2-tailed)	0.063	0.566	0.532	0.211	0.610	0.883	0.536	0.900	0.356	
	N	98	98	98	98	98	98	98	98	98	98

Furthermore, the results of neutrophils correlation gave a significant positive correlation with basophils and CRP ($p<0.05$)while a negative correlation with lymphocyte and ESR. Also, the results show that basophils gave a significant positive correlation with neutrophils, monocyte, and lymphocyte ($p<0.05$). A negative correlation between eosinophil and age, total WBCs, ESR, CRP, and D-dimer were demonstrated, while only a significant positive correlation with lymphocyte ($p<0.05$). The monocyte correlation shows significant positive with total WBCs count and basophils ($p<0.05$), negative correlation with ESR, and D-dimer. Lymphocyte correlation results represent a highly significant negative correlation with age ($p<0.01$), negative correlation with neutrophils, ESR, CRP, and D-dimer, and significant positive correlation with basophils and eosinophil ($p<0.05$). The ESR gave a significant positive correlation with age and CRP ($p<0.05$), negative correlation with total WBCs, neutrophils, monocyte, eosinophil, lymphocyte, and D-dimer. The CRP gave a highly significant positive correlation with age and ESR ($p<0.05$), a significant positive correlation with total WBCs and neutrophils ($p<0.05$), and a negative correlation with eosinophil and lymphocyte. Finally, the D-dimer results gave negative correlations with eosinophil, monocyte, lymphocyte, and ESR but they were not significant.

Table 2: The correlation between the levels of immunological parameters. *. Correlation is significant at the 0.05 level (2-tailed).**. Correlation is significant at the 0.01 level (2-tailed).

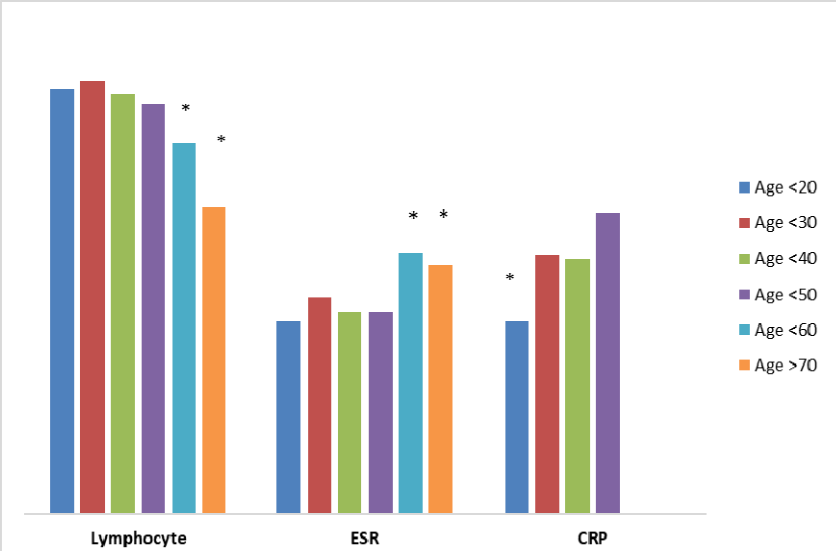

Figure 1: The correlation between the levels of immunological parameters and age. There is a significant increase in the levels of ESR and CRP with the escalation of age groups whereas the count of lymphocytes has been deceased reciprocally.

Discussion

Several studies have highlighted that the severity of COVID-19 infection is concomitant with the host immune response [1, 20, 21]. Meanwhile, it was companied by a wide range of changes in the peripheral blood immune-inflammation parameters. Therefore, this study was focus on the role of immunological laboratory data for early detection of COVID-19 patients and evaluate possible correlations between these parameters.

The results of the study revealed that most of the patient in the case study still within the normal range of total WBCs count, I suggest that this is because the COVID19 virus still in the progression stage due to the long incubation period of COVID19, maybe 14 days in most patients and as long as to reach 24 days in several cases. Furthermore, the laboratory finding taken directly after the admission of patients with positive PCR. And the small percentage with leukocytosis which indicates the second step were leukocytosis considered as one of the major immunological markers of SARS-CoV2 Patients. Henry et al., 2020 have indicated that onlyin SARS-CoV2 severe cases there wasanincrement in total WBC count [22].

The significant increase in total WBCs may signify clinical worsening and increased risk of a poor progression. Our results indicate that the increase in WBCs is driven by a highly significant positive correlation with neutrophils and monocytes, while a negative correlation with eosinophil and lymphocyte. It has been hypothesized that the survival of patients may be dependent on the ability of them to recover

lymphocytes which are killed by the virus. The increase of neutrophil in correlation with total WBCs counts indicate the intensity of the inflammatory response, while the decrease of lymphocyte counts suggests the damage tothe immune system. Around 5% of study case-patients have leukopenia which may result from immunosuppression or other inflammatory factors.

Likewise, COVID-19 positive patients with severe and fatal disease had significantly leukocytosis, and lymphopenia compared to non-severe disease and survivors [22]. In another study about the immune response in COVID-19 positive patientsbyQin et al. 2020, hereported that lymphopenia, leukocytosis as well as lower percentages of monocytes, eosinophil'sand basophils are notices in severe cases compared to mild cases.

One of the most common laboratory test abnormalities in COVID-19 patients is a decrease in lymphocyte. This suggests that lymphocyte may primarily site forthe attack of the COVID-19 virus, and lymphopenia may be used as a primary laboratory index in the diagnosis or to classify the severity of SARS-CoV-2 infections.Lymphopenia has been reported in patients with abnormal lung imaging findings compared to patients without lung involvement, so considerably lymphopenia might be a signature for severe COVID-19 infection [21].

Lymphopenia resulted from that both helper T cells and suppressor T cells were below normal levels, associated with severe cases. In severe cases,COVID-19 positive patients, a high percentage of naive helper T cells were reportedly increased, and memory helper T cells were decreased, also a lower level of regulatory T cells, which are more obviously damaged in severe COVID-19 positive patient's cases [18].The findings of our study are concomitant with a study conducted by Guan's et al, lymphopenia was reported in 83.2% of the admitted patients and diagnosed with SARS-CoV-2 infections as well as an increase of neutrophils and C-reactive protein was noticed (CRP)[3].

Novel findings propose that there is an essential role for eosinophilsagainst viral infections, an eosinophil-derived neurotoxin, and eosinophil cationic proteinsthat are released as granular proteins from eosinophilsduring single-stranded RNA infections. Moreover, eosinophil can produce nitric oxide and can activate cytotoxic-T cell proliferation which destroys virally infected cells[20]. According to all these facts, the eosinopenia in COVID-19 patients is of special interest[23, 24]. Compared to 10 COVID-19 patients with 30 patients affected by other viral pneumonia. They found lymphopenia and eosinopenia were common in COVID-19 patients compared to non-COVID-19 patients. We suggested that eosinopenia with lymphopenia may be an early potential indicator for COVID-19 with both diagnostic and prognostic value [25].

Compared to other parameters, there are normally very low % of eosinophil and basophils in healthy individuals, but decreased numbers have still been noticed in infections. Also in COVID-19, eosinopenia and basopenia were found[24]. High percent of COVID-19 patient results associated with a deficiency of basophils (basopenia), it may be due to a severe allergic reaction due to entrance of new virus to body and develop an infection which may take longer to heal.The mechanisms as to why basophils parameters tended to be reduced need to be investigated further, but they do agree with the finding of significant granulocyte reduction as in eosinopenia.

Furthermore, eosinopenia with high CRP could also effectively triage suspected patients with COVID-19 with fever[26]. The increase in eosinophil was also observed during treatment from the initial low levels and was used as a positive indicator of clinical improvement[10].We suggest that the main role of

eosinophil in COVID-19 patients is still unknown, it seems to have just diagnostic value on the COVID-19 pathology. Eosinopenia is a resulted of whether the block in eosinopoiesis also decreased the release of an eosinophil from bone marrow, or it may be supported viral clearance in the first stage of COVID-19 infection.

Because costly cytokine analysis is not routinely performed in most laboratories of the hospital, surrogate markers of CRP which an acute-phase reactive protein produced by the liver and release into the circulation as parallels to the severity of inflammatory response in patients with COVID-19. C-reactive protein (CRP) was increased especially in COVID-19 patients and increased of(CRP) is reported in the majority of severe cases COVID-19 patients. [7, 9, 27]. A previous study and a recent study have revealed its role in predicting of the early stageof SARS-COV-2 [9, 28].

The age of patients in this case study (9-77) years which give negative correlation with lymphocyte, highly significant negative correlation with eosinophil, and significant positive correlation with ESR and CRP which is means that old age COVID-19 patients mainly considered as more severe due to high level of lymphopenia and eosinopenia and high level of inflammatory response CRP and coagulation factor ESR.

All possible correlations of different immunological factors gives the same results of primary indicators of COVID-19 patients for significant lymphopenia, eosinopenia, and increase of pro-inflammatory biomarker CRP in the initial laboratory test.The increase of patient age increases the virulence stage and the severity of COVID-19 response. SARS-CoV-2 virus particles spread through the respiratory tract and infect other cells, inducing series of immune responses.

Our result suggeststhat total WBCs, neutrophils, monocyte, basophils, and ESR might not be affected with SARS-CoV-2 in the initial phase of the disease. It also suggests that SARS-CoV-2 might mainly act on lymphocytes, and eosinophil, and CRP as does SARS-CoV2. All case study is belonging to law-severity or moderate-severity cases of COVID-19 because all cases are dischargedd from the hospital at maximum of 28 days of admission with negative PCR.

Recommendation

Based on the findings of this study lymphocyte, eosinophil, basophil and CRP have very good accuracy in predicting cases with positive RT-PCR for COVID-19 in Jordan.

Limitation

The sample size was relatively small. Besides, since this study was conducted on blood laboratory parameters, not every patient was continuously monitored for all clinical manifestations.

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